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Molecular Events Following Gluten Stimulation In Coeliac Disease

A thesis submitted for the degree of Doctor of Philosophy

by

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Trinity College
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DECLARATION

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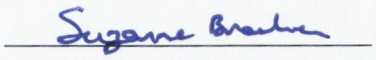

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ORAL PRESENTATIONS

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Abbreviations

Abbreviation	Meaning
aa	Amino acid
APC	Antigen presenting cell
ATP	Adenosine tri-phosphate
Bad	BCL2-antagonist of cell death
bcl-2	B-cell CLL/lymphoma 2
BLAST	Basic Local Alignment Search Tool
bp	Base pair
BSA	Bovine serum albumin
c-myc	Myelocytomatosis viral oncogene
CD	Cluster of differentiation
cDNA	Complementary DNA
COX	Cyclooxygenase
Ct	Threshold cycle
Cy3	Cyanine 3
DC	Dendritic cell
DMSO	Dimethyl sulfoxide
DTT	Dithiothreitol
DH	Dermatitis herpetiformis
EATL	Enteropathy associated T cell lymphoma
EDTA	Ethylene diaminetetra-acetic acid
ELISA	Enzyme linked immunosorbent assay
EMA	Endomysial antibody
FAM	6-carboxylfluorescein
FCS	Foetal calf serum
FF	Frazer's Fraction
FITC	Fluorescein isothiocyanate
FSC	Forward scatter
g	Gram
GALT	Gut associated lymphoid tissue
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GFD	Gluten free diet

GO	Gene ontology
HEV	high endothelial venule
HLA	Human leukocyte antigen
IBD	Inflammatory bowel disease
ICAM-1	Intercellular adhesion molecule 1
ICOS	Inducible co-stimulator
IEL	Intraepithelial lymphocyte
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IMS	Industrial methylated spirits
L	Ligand
l	Litre
LPL	Lamina propria lymphocyte
LPS	Lipopolysaccharide
M	Molar
MAdCAM-1	Mucosal addressin cell adhesion molecule 1
MAP	Mitogen-activated protein
MEK1	Mitogen-activated protein kinase kinase 1
MGB	Minor groove binder
MHC	Major histocompatibility complex
MICA	MHC class I polypeptide-related sequence A
min	Minute
ml	Millilitre
mM	Millimolar
MLN	Mesenteric lymph node
MMP	Matrix metalloproteinase
mol	Moles
mRNA	Messenger RNA
NF-κB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells
NK	Natural killer cell
nm	Nanometre
OU	Optical units
PBMC	Peripheral blood mononuclear cell

PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PE	Phycoerythrin
ppm	Parts per million
PT	Peptic tryptic
R	Receptor
Raf-1	v-raf-1 murine leukemia viral oncogene homolog 1
RCD	Refractory coeliac disease
RFU	Relative fluorescence units
RhoA	Ras homolog gene family, member A
RNAi	RNA interference
ROCK	Rho-associated, coiled-coil containing protein kinase
rRNA	Ribosomal RNA
RT	Room temperature
RT-PCR	Reverse transcription PCR
SC	secretory component
SDS	Sodium dodecyl sulphate
siRNA	Small interfering RNA
STAT	signal transducer and activator of transcription
SSC	Side scatter
SSC	standard sodium citrate buffer
TAMRA	6-carboxytetramethylrhodamine
TBE	Tris-Borate-EDTA
TCC	T cell clone
TCL	T cell line
TCR	T cell receptor
TFBS	Transcription factor binding site
TGase 2	tissue transglutaminase
TGF	Transforming growth factor
Th	T helper cell
TLR	Toll-like receptor
Tm	Melting temperature
TNF	Tumor necrosis factor
tTG	Tissue transglutaminase

Summary

Coeliac disease is a common autoimmune disorder, caused by an intolerance to dietary gluten in genetically predisposed individuals. Despite the advances in understanding of the nature of the disease, it remains unknown how gluten peptides breach the epithelium to initiate the inflammatory cascade and why certain gluten peptides trigger different aspects of the immune response. Investigations into gluten peptides have been conducted since the 1950s with the main focus examining the properties of wheat. In recent years, oats have received much attention due to opposing reports on their effect in coeliac patients. Barley and rye, although excluded from the official Codex Alimentarius gluten-free diet since the 1970s, have a lack of scientific evidence regarding their damaging effects.

It is known that antigen-presenting cells bearing the HLA molecules DQ2 or DQ8 can present gluten peptides to CD4+ T-cells in the gut mucosa, thus triggering an adaptive immune response. Approximately 95% of coeliac patients express HLA-DQ2 with the remainder generally expressing DQ8. The disease concordance rate for monozygotic twins is considered high at 75%. However, the concordance rate for HLA-matched dizygotic twins is only 17%, thus indicating that the HLA region only partially contributes to the development of the disease and other non-HLA genes must have a role. To date, no other disease susceptibility genes have been identified.

The objectives of this thesis were:

- To measure and compare the proinflammatory molecular response of coeliac intestinal tissue to wheat, barley and rye.
- To examine the role of the epithelial cell in the molecular processes of the gluten-induced proinflammatory environment of the coeliac gut lesion.
- To develop a high-throughput gene expression analysis system for hypothesis-free investigations .
- To validate this system and demonstrate the applicability of the results.

In the initial part of this study, proinflammatory cytokine production by coeliac and control intestinal tissue was measured following *ex vivo* stimulation with wheat, barley and rye prolamins (the alcohol-soluble fraction of gluten). TaqMan RT-PCR was used to quantify cytokine mRNA and secreted cytokine protein was measured in the culture

supernatant by enzyme-linked immunosorbent assay. All three cereals induced an up-regulation of IFN-γ mRNA but not of IL-2 mRNA or the protein of either cytokine, in coeliac tissue when compared to control tissue. Barley caused the greatest increase in IFN-γ mRNA while rye and wheat induced similar levels. These findings provide evidence that like wheat, barley and rye cause immune activation in the mucosa of coeliac patients and support the practice that barley and rye should be excluded from the gluten-free diet. Using this experimental set-up, IFN-γ mRNA proved to be the only informative marker of gluten-induced immune activation. A limitation of this system is the restriction on the number of molecular responses that can be assessed. Therefore a microarray system was developed for high-throughput gene expression analysis with a hypothesis-free approach.

In the next part of this study, the gene expression profile of intestinal epithelial cells of coeliac patients ingesting a gluten-containing diet was compared to that of non-coeliac control patients also consuming a gluten-containing diet, using microarray analysis. The results showed a number of genes which had significantly altered expression in active coeliac disease. Functional analysis on these genes revealed a number of processes to be disordered in active coeliac disease such as proliferation, differentiation, survival and apoptosis.

In the final part of this study, the microarray analysis system was validated, to assess the reproducibility of the results obtained. A number of genes found to have regulated expression with microarray analysis were investigated using TaqMan RT-PCR. The majority (80%) of these were corroborated, demonstrating the high quality of the microarray system used. Follow-up analysis of the protein expression of two of these genes showed that the increased levels of mRNA observed with microarray analysis were translated into increased levels of protein.

The findings of this thesis provide insight into the molecular processes that occur in the coeliac gut in response to gluten.

Chapter 1

General Introduction

1.1 Intestinal Immune System

1.1.1 Features of the Intestinal Immune System

1.1.1.1 Gut Associated Lymphoid Tissue.

The mucosal associated lymphoid tissue (MALT) is a secondary or peripheral lymphoid organ found in the mucosal tissue of respiratory, urogenital or gastrointestinal tracts. Its complexity can range from the simple, loose clusters of lymphoid cells with little organisation found in the lamina propria of intestinal villi to organised structures such as the tonsils, appendix and Peyer's patches. In the gut, this immune defence system is known as the gut-associated lymphoid tissue (GALT), and it is estimated that GALT constitutes approximately 40% of the body's immune effector cells [O'Dorisio 1986] (Fig.1.1.1).

Peyer's patches are the best-studied structure of the GALT. The epithelium covering the dome of the Peyer's patch is comprised of cuboidal epithelial cells which express class II MHC antigens, few goblet cells and specialised antigen-sampling cells called M (microfold) cells [Doe 1989]. M cells can sample food and microbe antigens and transport them across the epithelium to the underlying B-cells, T-cells, macrophages and dendritic cells. Within the Peyer's patch, there are large follicular structures enriched in B cells. These B cells are predominantly IgM-bearing although there is a significant proportion displaying surface IgA and committed to IgA synthesis. Adjacent to the follicles, there are parafollicular regions which are enriched for T cells predominantly of the helper-induced subset (Th). These regions also possess specialised high endothelial postcapillary venules [Simecka 1998].

1.1.1.2 Epithelium

The small intestine is lined by a continuous one-cell thick sheet of epithelial cells consisting mainly of columnar polarised enterocytes. Interspersed among these cells are mucous-producing goblet cells, endocrine cells and intraepithelial lymphocytes (IELs). A number of pluripotent stem cells are anchored within the lower crypt of the mucosa. These give rise to a continuous supply of undifferentiated enterocytes which differentiate and mature as they migrate up to the tip of the villous, where they are eventually sloughed off. The mature enterocytes function as absorptive cells as well as

a physical barrier. Additionally, enterocytes can function as immune cells. Firstly, they constitutively express MHC class II proteins [Bland 1988] and during inflammation, this expression is extended from just the villous tip to the entire epithelium [Kelly *et al.* 1988]. Secondly, enterocytes have been shown to produce their own cytokines, which they secrete in response to injury or infection and to regulate the function of other cells [Stadnyk 1994] (section 1.1.2, Cytokines).

The basal side of enterocytes rests on a fibrous basement membrane and on the apical side the intestinal brush border extends into the lumen. The brush border is comprised of numerous microvilli that increase the intestinal surface area approximately 600-fold which over-lie a transverse fibrillar cytoskeleton called the terminal web [Holmes and Lobley 1989]. The functions of the brush border are digestion, transport and regulation. The M cells of the Peyer's patches differ from enterocytes by lacking a brush border [Neutra *et al.* 1988].

The continuity of the epithelium is maintained by tight junctions (zona occludens) and by actin-rich connections (zona adherens and zona maculens) which join the uppermost portions of the cells. Intestinal transport may be paracellular, via the tight junctions, or transcellular, where the molecules are moved through the epithelial cells either by active or passive transport.

Within the normal epithelium there is a large population of lymphocytes, termed intra-epithelial lymphocytes (IELs). The majority (90%) of IELs are $CD3^+$ T-cells and of these, approximately 80% express CD8 with the remainder being $CD4^+$ or $CD4^-CD8^-$ [Simecka 1998]. IELs mainly have an αβ T-cell receptor (TCR) though a significant proportion does express γδ TCR. In health, approximately 5% of IELS are γδ-TCR positive but this proportion can increase to 30% in inflammatory conditions such as coeliac disease [Viney *et al.* 1990]. $\alpha\beta^+$ lymphocytes migrate from the Peyer's patches, differentiate in the thymus and recirculate to the epithelium. In contrast, $\gamma\delta^+$ lymphocytes migrate from the bone marrow as immature cells and differentiate in the gut microenvironment [Guy-Grand *et al.* 1991]. $CD8^+$ T-cells are known to have a cytotoxic function with the ability to lyse virus-infected cells; thus IELs presumably participate in the resistance to and recovery from viral infections. Although less

prominent, $CD4^+\alpha\beta^+$ IELs exist and have a helper function. They have been shown to produce Th2 cytokines which participate in B-cell activation and maturation [Fujihashi *et al.* 1993]. It is thought these cells may become activated after presentation of luminal antigens by epithelial cells [Simecka 1998]. γδ-T cells are involved in regulating growth and differentiation of epithelial cells [Komano *et al.* 1995]. In culture, γδ-T cells have been shown to produce a cytokine, keratinocyte growth factor, that supports growth of epithelial cells [Boismenu and Havran 1984].

1.1.1.3 Lamina Propria

The Lamina Propria, a mesh of connective tissue that underlies the gut epithelium, contains a large and heterogeneous group of lymphoid and myeloid cells. These cells include lymphocytes, macrophages, dendritic cells, neutrophils, and mast cells. Similar to peripheral blood, a large percentage (60-70%) of lamina propria T-cells are $CD4^+$ and the majority of these appear to be TCR $\alpha\beta^+$ [Brandtzaeg *et al.* 1998]. Lamina propria T-cells differ from peripheral blood by being in a more activated state and express CD25 and HLA-DR antigen. These cells have a mature or memory phenotype as indicated by their surface markers $CD44^{high}$, $CD62^{low}$, $CD45RO^+$ and high levels of the integrin $\alpha_4\beta_7$ [James *et al.* 1986; Schieferdecker *et al.* 1992]. The $CD4^+$ subset is predominantly Th2-like but Th1 cells also exist [Simecka 1998]. The high levels of Th2 cells help the B-cell production of IgA. Lamina propria T-cells have been demonstrated to produce IL-2, IL-4, IL-5, IL-10 and IFN-γ. T cell lines derived from this population show varying types and amounts of cytokines produced [Braunstein *et al.* 1997; Harriman *et al.* 1992]. Although a significant number of IFN-γ-producing CD4+ T cells are present in the lamina propria, IL-5-producing T cells have been shown to be more frequent [Taguchi 1990].

Lamina propria B-cells have migrated from the Peyer's patches to the mesenteric lymph node and are recirculated back to the lamina propria where they develop into plasma cells. Plasma cells are terminally differentiated, non-dividing effector cells which synthesize and secrete antibodies. In the small intestine, approximately 80% of the total plasma cells secrete IgA antibodies (section 1.1.2.4), while approximately 18% secrete IgM and 3% secrete IgG [MacDonald 2003]. Lamina propria B-cells are

regulated by T-cell production of cytokines (section 1.1.2.3) which aid in terminal differentiation and isotype switching.

1.1.2 Processes within the Intestinal Immune System

1.1.2.1 Antigen Presentation

In T-cells, antigen recognition is mediated by a membrane-bound receptor called the T-cell receptor (TCR). The TCR, in complex with accessory molecules, can only recognise antigen when associated with a cell-membrane molecule known as the major histocompatibility complex (MHC), a structure that is expressed on the surface of antigen-presenting cells (APCs). The MHC, termed the HLA complex in humans, is organised into three regions based on the type of molecules encoded. The class I molecules, in humans, are encoded by HLA-A, HLA-B and HLA-C loci, while the class II molecules are encoded by the HLA-D region which is further divided into HLA-DQ, HLA-DR and HLA-DP sub-regions. Class III MHC molecules include a diverse group of non-membrane proteins that appear to play no role in antigen presentation. X-ray crystallographic analysis has revealed that the peptide-binding region in both class I and class II molecules is a deep cleft with a floor formed by eight anti-parallel β-sheets and sides formed by two anti-parallel α-helices. The structure of the antigen binding cleft is determined by the polymorphic alleles encoding class I and class II molecules thus creating diversity in the ability to recognise a wide range of antigens [Kuby 1994].

Class I molecules are present on nearly all nucleated cells whereas class II molecules are only expressed on APCs such as macrophages, dendritic cells and B-cells. Endogenous antigen, for example viral proteins synthesised within virus-infected cells, are degraded into peptide fragments that bind to class I molecules within the endoplasmic reticulum. The antigen-MHC complex is then transported to the cell membrane where it is recognised only by $CD8^+$ T-cells. These T-cells function as cytotoxic cells. Exogenous antigen, which is produced outside the cell, is taken into the APC by endocytosis or phagocytosis. The antigen is then broken down into peptide fragments within the endocytic-processing pathway and the fragments bind to the cleft of the MHC class II molecule prior to exportation to the cell surface. T-cells

expressing CD4 can only recognise antigen that is associated with class II molecules. These T-cells function as helper cells by secreting cytokines [Kuby 1994].

The main route for particulate antigen to gain access to the intestinal immune system is thought to be through the M cells [Bilsborough and Viney 2004]. In the Peyer's patch, there are many APCs such as macrophages, dendritic cells and B-cells, though it is the dendritic cells (DCs) that are considered the main force in initiating T-cell responses [Wittig and Zeitz 2003]. DCs in the subepithelial dome process antigen as relative immature cells and then migrate to the T-cell region and present antigen to naïve T-cells. At this stage they have the properties of mature DCs with high surface expression of co-stimulatory molecules such as CD40, CD80, CD86 and adhesion molecules such as CD44 and intercellular adhesion molecule 1 [Kelsall 1999]. DCs are also active in the lamina propria where they are distributed along the entire intestinal epithelium. They have been shown to sample antigen from the gut lumen by extending their dendrites through the epithelial cells of the gut barrier. The barrier integrity is conserved by the expression and modulation by DCs of tight junction proteins, including junctional adhesion molecule (JAM), occludin, claudin 1 and zona occludens 1 [Rescigno *et al.* 2001].

The ability to distinguish between pathogens and commensals is necessary for the maintenance of intestinal homeostasis. It has been proposed that DCs use their innate toll-like receptors (TLR) and C-type lectins to distinguish between microbial-associated molecular patterns (MAMP) expressed by commensal bacteria and pathogen-associated molecular patterns (PAMP) expressed by pathogenic bacteria [Granucci and Ricciardi-Castagnoli 2003]. TLRs are transmembrane proteins that recognise specific microbial patterns to direct the ensuing immune response (see Table 1.1.1) [Akira and Takeda 2004]. C-type lectins preferentially bind carbohydrate antigens and are associated with antigen uptake, including virus, bacteria, parasites, and yeasts [Figdor *et al.* 2002]. NOD (nucleotide-binding oligomerization domain) proteins are another recently discovered family of innate cytosolic proteins which function primarily to recognise the peptidoglycan of bacterial cell walls. Nod1 is found in epithelial cells while Nod2 can be expressed by dendritic cells, macrophages and epithelial cells. Both proteins activate the NF-κB pathway and a mutation in Nod2 has

Receptor	Ligand	Origin of ligand
TLR1	Triacyl lipopeptides	Bacteria and mycobacteria
	Soluble factors	*Neisseria meningitidis*
TLR2	Lipoprotein/lipopeptides	Various pathogens
	Peptidoglycan	Gram-positive bacteria
	Lipoteichoic acid	Gram-positive bacteria
	Lipoarabinomannan	Mycobacteria
	Phenol-soluble modulin	*Staphylococcus epidermis*
	Glycoinositolphospholipids	*Trypanosoma cruzi*
	Glycolipids	*Treponema maltophilum*
	Porins	*Neisseria*
	Atypical lipopolysaccharide	*Leptospira interrogans*
	Atypical lipopolysaccharide	*Porphyromonas gingivalis*
	Zymosan	Fungi
	Heat-shock protein 70	Host
TLR3	Double-stranded RNA	Viruses
TLR4	Lipopolysaccharide	Gram-negative bacteria
	Taxol	Plants
	Fusion protein	Respiratory syncytial virus
	Envelope protein	Mouse mammary-tumour virus
	Heat-shock protein 60	*Chlamydia pneumonia*
	Heat-shock protein 70	Host
	Type III repeat extra domain A of fibronectin	Host
	Oligosaccharides of hyaluronic acid	Host
	Polysaccharide fragments of heparan sulphate	Host
	Fibrinogen	Host
TLR5	Flagellin	Bacteria
TLR6	Diacyl lipopeptides	*Mycoplasma*
	Lipoteichoic acid	Gram-positive bacteria
	Zymosan	Fungi
TLR7	Imidazoquinoline	Synthetic compounds
	Loxoribine	Synthetic compounds
	Bropirimine	Synthetic compounds
	Single-stranded RNA	Viruses
TLR8	Imidazoquinoline	Synthetic compounds
	Single-stranded RNA	Viruses
TLR9	CpG-containing DNA	Bacteria and Viruses
TLR10	not determined	not determined
TLR11	not determined	Uropathogenic bacteria

Table 1.1.1: Toll-like receptors and their ligands.
(from Akira and Takeda 2004).

been associated with Crohn's disease [Girardin and Philpott 2004]. DCs are unique as APCs in their ability to present antigen to both naïve and primed T-cells, whereas macrophages and B-cells are only effective in presenting antigen to primed T-cells [Simecka 1998]. Epithelial enterocytes have been shown to express HLA-DR, though not the co-stimulatory molecules CD80 and CD86 required for activation of naïve T-cells [Cheroutre and Madakamutil 2004]. Thus antigen from the gut lumen can be taken up by these epithelial cells and presented directly to primed T-cells or to local DCs which can mature and present antigen to naïve T-cells [Cheroutre and Madakamutil 2004].

Once stimulated, the T-cells in the Peyer's patches can provide help for the B-cell switch to IgA synthesis, a process that is completed in the germinal centre.

1.1.2.2 Lymphocyte Migration

After initial exposure to antigen, proliferation and partial differentiation, the primed T- and B-lymphocytes leave the Peyer's patches or other mucosal inductive sites and migrate via the mesenteric lymph node and peripheral blood before homing to the lamina propria or mucosal epithelium [Simecka 1998]. Here, they perform their effector activities. The preferential migration of stimulated mucosal lymphocytes to mucosal tissues is mediated by the expression of adhesion molecules on lymphocytes and cells associated with mucosal tissues. It is the interaction of these adhesion molecules with their ligands on high endothelial venules (HEVs) or epithelial cells that directs the migration of lymphocytes. A unique adhesion molecule, known as mucosal addressin cell adhesion molecule 1 (MAdCAM-1) is involved in lymphocyte trafficking into mucosal tissues. MAdCAM-1is a glycoprotein found on HEVs of Peyer's patches, mesenteric lymph nodes and vascular endothelium of the lamina propria, but not on HEV of peripheral (non-mucosal) lymph nodes [Streeter *et al.* 1988]. Circulating lymphocytes recognise HEV through the expression of MAdCAM-1 which causes the lymphocytes to bind and migrate into mucosal lymphoid tissue. Lymphocytes binding to MAdCAM-1 express two major adhesion molecules, L-selectin and $\alpha 4\beta 7$ integrin. L-selectin is present on small, mature lymphocytes, monocytes and neutrophils. L-selectin does not, however, recognise the form of MAdCAM-1 found on the endothelium in the lamina propria as this form lacks the L-

selectin-binding carbohydrates [Berg *et al.* 1993]. Consequently, L-selectin probably is not involved in the homing of lymphocytes to normal intestinal mucosa, but it is likely to be involved in the circulation of lymphocytes into Peyer's patches. In humans, α4β7 is known as Lymphocyte Peyer's patch adhesion molecule-1 (LPAM-1). It is expressed on lymphocytes and monocytes and its expression increases upon cell activation. α4β7 can also bind fibronectin and VCAM-1 [Postigo *et al.* 1993]. It appears that homing of primed GALT-derived lymphocytes to human gut mucosa is determined mainly by their high levels of α4β7 in the absence of L-selectin [Bargatze *et al.* 1995]. Human intestinal lamina propria B-cell blasts, as well as other intestinal mononuclear cells, express high levels of α4β7 but little or no L-selectin [Salmi *et al.* 1994; Farstad *et al.* 1995]. DCs of the GALT can mediate up-regulation of the expression of α4β7 on lymphocytes [Stagg *et al.* 2002].

Chemokines also regulate the directional trafficking of lymphocytes and endothelial cell activation critical to lymphocyte migration [Dwinell *et al.* 2003]. After the adhesion molecules have tightly bound the lymphocytes, chemoattractants direct the migration of the lymphocytes which crawl along the endothelium where chemokines are deposited in a solid phase, and move into the tissue along an immobilised gradient of chemotactic signals [Brandtzaeg *et al.* 1999]. Small intestinal epithelial cells abundantly express CCL25 and its receptor, CCR9, is expressed by almost all small intestinal lymphocytes in humans [Kunkel *et al.* 2000]. DC migration is thought to be largely under the control of chemokines and chemokine receptors [Bilsborough and Viney 2004].

1.1.2.3 Cytokine Production

Cytokines are a diverse group of hormone-like polypeptides which bind to specific receptors on the membrane of target cells, modulating an immune response by a variety of pathways. Many different cells secrete cytokines such as T-cells, B-cells, monocytes, macrophages, dendritic cells, natural killer (NK) cells, fibroblasts, stromal cells, enterocytes, endothelial cells and platelets, and their effects can differ depending on the target cell [Kuby 1994b]. Cytokines bind to specific receptors and are extremely potent at low concentrations. Their potency is due to series of cascades and networks where one cytokine can cause the secretion of another, resulting in amplification loops.

Cytokines can also inhibit other cytokines and cause their own negative feedback [Stadnyk 1994].

T helper cells are key mediators of humoral and cell-mediated immune responses involved in mucosal immunity. They exert their helper functions through secretion of cytokines, which either act on the cells that produce them in an autocrine manner or modulate the responses of other cells through paracrine pathways [Kuby 1994b]. T helper cells are roughly separated into two cell subpopulations, Th1 and Th2 [Mosmann and Coffman 1989]. The Th1 subset is responsible for classical cell-mediated functions such as activation of macrophages and cytotoxic T-cells as well as delayed-type hypersensitivity. Th1 cells secrete proinflammatory cytokines such as interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), TNF-β (also called lymphotoxin), interleukin-2 (IL-2) and granulocyte-macrophage colony-stimulating factor (GM-CSF). The Th2 subset has a role in humoral immunity, stimulating the production of IgM, IgE and IgA and enhancing eosinophil proliferation and function. Th2 cells secrete IL-4, IL-5, IL-6 and IL-13 [Kuby 1994b]. The differentiation from naïve or precursor T cells progresses through a Th0 intermediary cell stage which upon repeated stimulation differentiates further into either a Th1 or Th2 cytokine phenotype. Different types of APC may selectively induce either Th1 or Th2 responses and the type of costimulatory molecule on APCs may determine the phenotypic responses [Wittig and Zeitz 2003]. CD40 has been shown to induce a Th1 cytokine response by production of IL-12 in macrophages and DCs. The CD80 (B7.1) and CD86 (B7.2) ligands on activated B-cells play distinct roles in differentiation of Th1 or Th2 type cells [Kuchroo *et al.* 1995]. The type of antigen and its concentration also influences the differentiation of T-cells.

Another division of T helper cells exists, often called T suppressor or T regulatory cells. There are two well-characterised types of regulatory T-cells: the first type termed Tr1 cells produce IL-10, some IL-5 and IFN-γ with or without TGF-β, but little or no IL-2 or IL-4. They proliferate poorly following TCR-mediated activation and functional studies have demonstrated that these cells have immunosuppressive properties and have been shown to prevent the development of Th1-mediated autoimmune diseases [Groux *et al.* 1997]. The second type, termed Th3 cells produce

IL-4 and TGF-β and have been shown to have properties that can inhibit the effects of pathogenic autoreactive T cells [Chen *et al.* 1994]. Th3 and Tr1 cells are induced by semi-mature dendritic cells under the influence of regulatory cytokines including IL-10, TGF- β and IL-4. They are capable of suppressing Th1 and Th2 responses and function in infection to limit pathogen-induced immunpathology [Mills and McGuirk 2004].

Recently, it has been realised that intestinal epithelial cells are not just affected by cytokines but are major producers of cytokines themselves. Because they are found at the interface between the organism and environment, they are in a position to signal changes in the environment. Epithelial cells may constitutively express cytokines or they can be induced to make cytokines by interaction with other cytokines [Stadnyk 1994]. Some of the important cytokines in gut immune responses and the cells that produce them are detailed in table 1.1.2.

1.1.2.4 Secretory Immunoglobulin A

The characteristic antibody of mucosal immunity is immunoglobulin A (IgA), and secretory IgA (sIgA) is the dominant functional antibody present in mucosal secretions. More IgA is secreted into the gut (approx.40mg/kg) everyday than the total daily production of IgG (approx. 30mg/kg) [Conley and Delacroix 1987]. sIgA is made up of two IgA monomers joined by a covalently linked peptide called the J-chain (joining chain), which is bound to a glycopeptide known as secretory component (SC). B-cells in the lamina propria secrete dimeric IgA which is then transported to the epithelial cells. Secretory component is synthesised by epithelial cells and resides on their basal surface. It acts as a receptor for dimeric IgA and once bound to SC, IgA is transported through the epithelial cell and released into the mucosal secretions. The SC remains bound to the IgA stabilising its structure and increasing its resistance to proteolytic digestion [Tomasi 1970].

The main function of sIgA is to protect the mucosal barrier from foreign antigens including food antigens, bacteria, viruses and toxins. sIgA can block the access of potentially allergenic molecules derived from food or drugs [Doe 1989]. Secretory IgA binds to viral surface proteins thus preventing the virus from infecting the host cell. In

Cytokine	Cell source	Function
IFN- α	Leukocytes	Stimulates macrophages ad NK cells. Inhibits viral replication.
IFN-γ	Th1 cells, NK cells	Macrophage activator, capable of increasing MHC class II expression, enhancing phagocytosis and cytotoxicity, and inducing further secretion of cytokines.
TNF-α	Th1 cells, macrophages, epithelial cells	Pro-inflammatory effect, promotes IL-1, IL-6 and IL-8. Increases paracellular permeability via effect on tight junctions.
TGF-β	Regulatory T-cells, macrophages	Suppresses proliferation of lymphocytes and has significant role in oral tolerance. Promotes B-cells to switch antibody class expression to IgA.
IL-1	Th1 cells, dendritic cells, macrophages, epithelial cells	Pro-inflammatory cytokine, activates resting T cells and macrophages, stimulated synthesis of IL-2 and IFN-γ
IL-2	Th1 cells	Pro-inflammatory cytokine, enhances secretion of other cytokines, induces expression on class II molecules
IL-3	Epithelial cells, NK cells, mast cells	Stimulates growth and differentiation of eosinophils and B cells.
IL-4	Th2 cells, NK cells	Main promoter of Th2 cells. Activates B-cells and promotes isotype switching to IgA or IgE in response to antigen exposure. Can also function as an anti-inflammatory cytokine, by inhibiting the secretion of pro-inflammatory cytokines.
IL-5	Th2 cells	Increases the production of IgA from IgA^+ B-cells. Promotes differentiation of IgA^+ B-cells into IgA plasma cells.
IL-6	Th2 cells, macrophages, epithelial cells	Promotes the differentiation of B cells to plasma cells, increases IgA secretion, enhances maturation of T cells.
IL-7	Epithelial cells, stromal cells	Extra-thymic regulator of γδ-IEL differentiation and growth. Stimulates proliferation of lamina propria lymphocytes (LPLs), enhances their cytokine release .
IL-8	Epithelial cells, macrophages	Pro-inflammatory cytokine, recruits pro-inflammatory cells such as neutrophils.
IL-10	Th2 cells, Regulatory T-cells	Acts on macrophages to prevent their activation and the induction of pro-inflammatory cytokines, thus inhibiting T-cell recruitment into the intestine. Can also stimulate B-cell differentiation.
IL-12	B cells, activated macrophages	Principal cytokine in promoting development of Th1 cells. Defends against intracellular mucosal pathogens.
IL-15	Epithelial cells, monocytes, T cells.	Activates and expands memory/effector T cells. Chemoattractant for T cells
IL-18	Activated macrophages, epithelial cells	Induces IFN- γ production, role in developing IELs.

Table 1.1.2: Cytokines- source and function. (Adapted from Kuby 1994b; Feghali and Wright 1997; Simecka 1998)

the same way, bacteria are neutralised when sIgA binds to adhesins, such as those found on pilli, preventing the bacteria from binding to the epithelial cell [Williams and Gibbons 1972]. Bacterial toxins can also be inactivated by sIgA. It is thought IgA can inhibit assembly of some viral particles within epithelial cells while the antibody is being transported across the epithelium [Mazanec *et al.* 1995]. Antigen that crosses the epithelial barrier can be eliminated by sIgA by complexing with it and transporting it back across the epithelium [Lamm *et al.* 1991].

1.1.2.5 Oral Tolerance

Upon encountering an antigen, the immune system can either develop an immune response or enter a state of specific unresponsiveness called tolerance. The purpose of inducing tolerance is to prevent autoimmune responses against self-antigens, a process which mostly involves deletion of self-reactive T-cells in the thymus during the foetal and perinatal periods [Schwartz 1989]. However, after these periods, tolerance can also be induced in the periphery and one of the most effective means of inducing peripheral tolerance is oral tolerance. Oral tolerance is defined as the induction of a state of systemic immune non-responsiveness to orally administered antigen upon subsequent antigen challenge [Wittig and Zeitz 2003]. The main mechanisms of oral tolerance are clonal deletion, anergy and active suppression [Weiner 1997]. Deletion is through a process of "programmed cell death", known as apoptosis. Anergy is the inactivation of cells to respond to antigen, thought to occur when antigen is presented by an APC which lack co-stimulatory molecules such as CD80 and CD86. Active suppression occurs when T-cells secrete immunosuppressive cytokines such as TGF-β, IL-10 and IL-4 in response to antigen. Oral tolerance mediated by anergy or clonal deletion occurs preferentially at high doses of antigen [Friedman and Weiner 1994]. Oral feeding of high doses results in the loss of antigen-reactive T-cells from Peyer's patches, at least some occurring through apoptosis [Chen *et al.* 1995]. Anergy of $CD4^+$ T cells also occurs at high doses [Hirahara *et al.* 1995] but different T helper cell populations vary in their sensitivity. Th1 cells are tolerised at lower doses of antigen than Th2 cells [Friedman 1996]. Oral tolerance due to active suppression occurs at low doses of antigen. Oral feeding of low doses leads to an increase in the numbers of T-cells secreting TGF-β, IL-10 and IL-4, although IL-10 and IL-4 have been shown not to be necessary for oral tolerance [Simecka 1998-]. TGF-β has been shown to be of

great importance in establishing and maintaining oral tolerance and neutralisation of this cytokine leads to a break in tolerance [Miller *et al.* 1992; Haneda *et al.* 1997]. The β-chemokine, MCP-1 [Karpus and Lukacs 1996] and IFN-β [Nelson *et al.* 1996] have also been found to be important in promoting oral tolerance while IL-12 has been demonstrated to be capable of preventing oral tolerance [Marth *et al* 1996].

A breakdown of oral tolerance may be the cause of the abnormal immune response to gluten found in coeliac disease, which leads to the development of a hypersensitivity reaction in the gut [Wittig and Zeitz 2003].

1.2 Introduction to Coeliac Disease

Coeliac disease is an inflammatory disease which results from sensitivity to ingested dietary gluten in genetically pre-disposed patients [Kennedy and Feighery 2000]. The immune response to gluten in the gut contributes to small intestinal mucosal inflammation, potentially causing nutrient malabsorption and excessive stimulation of the gut-associated immune system [Cuoco *et al.* 1998].

1.2.1 History of Coeliac Disease

The first description of childhood and adult coeliac disease was written in the second half of the second century A.D. by Aretaeus of Cappadocia [Cooke and Holmes 1984]. It was he who gave the word coeliac to persons suffering from this condition. The term comes from the Greek word koiliakos which meant suffering in the bowels and when translated into Latin the word became coeliac. In 1888, Samuel Gee reported the "Coeliac Affection". He described the disorder as one which is found in persons of all ages but especially children, and gives rise to loose, watery, bulky, pale stools. He also noted that patients suffered from emaciation and cachexia as well as diarrhoea. His comment "if the patient can be cured at all, it must be by means of diet" was particularly pertinent, and he recommended a diet avoiding highly starchy food. Poynton *et al,* 1914, were the first to correlate pathological and clinical findings in coeliac disease when they noted the jejunum "showed marked small round-celled infiltration, prominence of solitary lymph follicles, and congestion and haemorrhage onto the free surface of the section" during autopsy of a nine-year old boy. Poynton also noted one patient who could not consume bread and butter without exacerbating symptoms of the disease [Cooke and Holmes 1984]. In the following years many recommendations were made regarding what should be included or excluded in the diet, none of which proved to be conclusive. It was not until 1950 that Dicke proved that removal of wheat from the diet caused the symptoms and signs of coeliac disease to disappear, and that the harmful factor occurred in wheat flour but not wheat starch [Dicke 1950]. Paulley [1954] was the first to describe small-bowel mucosal villous atrophy with chronic inflammation as a feature of coeliac disease, which he observed from surgery of coeliac patients. With the introduction of peroral intestinal biopsies in 1957 [Shiner], a diagnosis of coeliac disease on the basis of histology became the standard.

1.2.2 Clinical Presentation

The classical clinical symptoms of coeliac disease are diarrhoea, weight loss, weakness, and in children, failure to thrive [Kennedy and Feighery 2000]. Severe malabsorption is often a feature of this disease. However in recent years, the symptoms have decreased with milder, less obvious symptoms prevailing. In children, mild gastrointestinal symptoms and signs of nutritional deficiencies should suggest possible coeliac disease [Visakorpi and Maki 1994]. In adults, tiredness, moderate weight loss and mild abdominal symptoms may be the only signs of coeliac disease [McElvaney *et al.* 1992]. Atypical features such as dental enamel defects [Aguire *et al.*1997], recurrent oral aphthous ulceration [O'Farrelly *et al.* 1991], infertility or miscarriages may be suffered by patients with coeliac disease [Collin *et al.* 1996]. Chronic malabsorption frequently leads to nutritional deficiencies in coeliac patients. Osteoporosis and iron-deficiency anaemia are the most common. Deficiencies of magnesium, zinc, copper, selenium, thiamin, niacin, pyridoxine, folic acid, vitamin B12, and vitamins A, D, E, and K, have been reported [Kennedy and Feighery 2000].

The iceberg model has been used to describe the spectrum of coeliac disease [Farrell and Kelly 2001] with the "tip" of the iceberg consisting of patients with classical coeliac disease and the larger "submerged" portion being made up mostly of patients with silent coeliac disease. The "base" of the iceberg consists of a small group of patients with latent coeliac disease. Silent coeliac disease is characterised by mucosal inflammation as seen in classical coeliac patients but with absent, very mild or atypical symptoms while latent coeliac disease is the term generally given to patients with positive antibodies but normal or near-normal mucosa who may go on to develop overt coeliac disease at a later date.

In the gut, coeliac disease is recognised by a characteristic lesion featuring villous atrophy, crypt hyperplasia and increases in intra-epithelial lymphocyte (IEL) and lamina-propria lymphocyte (LPL) density [Oberhuber *et al.* 1999]. Enterocytes may decrease in height and become more cuboidal in shape. The stages in development of the coeliac lesion were originally described by Marsh and Crowe [1995]. Oberhuber *et al.* [1999] have modified the stages to allow for a more concise definition. Type 0 is described as normal mucosa with less than 40 IELs per 100 enterocytes. Type 1 is

described as the infiltrative type with normal villous architecture, normal crypt height but an increase in IELs of more than 40 per 100 enterocytes. Type 2 is termed the hyperplastic type, characterized by normal villous architecture, an increase in IELs but with crypt hyperplasia. Type 3, divided into three separate classes, is called the destructive type of lesion. The first class, 3a, is depicted as an increase in IELs, crypt hyperplasia and mild villous atrophy where mild or moderate blunting of the villi exists. Class 3b differs by having marked villous atrophy where only short tent-like remainders of the villi exist. Class 3c further differs by having total villous atrophy where the mucosa is flat and no more villi are recognized.

1.2.3 Diagnosis

Several serological screening tests are now available. The first test- for anti-gliadin antibodies- has been in use since the 1970s but with a sensitivity of 80% and a specificity of approximately 85%, this test has now been superceded by better tests [Rostom *et al.* 2005]. Antiendomysial antibodies (EMA) were first tested for in 1983 [Chorzelski *et al.*] and with a sensitivity of 90% and specificity of 99% has remained the gold standard in serological testing of coeliac disease [Cerf-Bensussan *et al.* 2003]. With the discovery of tissue transglutaminase (TGase 2) as the autoantigen in coeliac disease in 1997 [Dieterich *et al.*1997], antibody testing against TGase 2 began. The IgA antihuman Tgase 2 has been found to have a sensitivity of 93% and specificity of 95% [Cerf-Besussan *et al.* 2003].

Although IgA antibodies are more sensitive [Rostom *et al.* 2005], 2% of patients with coeliac disease are IgA-deficient; thus, IgG testing needs to be carried out routinely to identify these patients. Another problem associated with serological screening is that titres of EMA correlate with the degree of villous atrophy [Sategna-Guidetti *et al.* 1993] and presentation of symptoms [Tursi *et al.* 2001]. Approximately 25% of patients with subtotal villous atrophy may be missed with serological screening [Rostami *et al.* 1999; Dickey *et al.* 2000].

A definitive diagnosis of coeliac disease should only be made when histological evidence of small intestinal abnormalities is found [Walker-Smith *et al.* 1990]. Originally, a biopsy capsule, such as the Crosby or Watson capsule, which employs a

suction-guillotine mechanism, was used to take biopsies from the jejunum near the ligament of Treitz. More recently, biopsies are obtained endoscopically, particularly in adults. Biopsies are taken from the second part of the duodenum, and multiple biopsies are required to make allowances for the generally small size of the specimens. A follow-up histological examination is usually carried out approximately one year following treatment to ensure mucosal recovery is occurring [Oberhuber *et al.* 1999].

1.2.4 Treatment

The current treatment for coeliac disease is a permanent withdrawal of gluten from the diet [Walker-Smith *et al.* 1990]. This entails avoiding foods containing wheat, barley and rye. Oats are generally considered to be non-toxic to coeliac patients [Srinivasan *et al.* 1996; Janatuinen *et al.* 2002; Thompson *et al.* 2003]; however, there is a difficulty in guaranteeing commercially available oats are free from contamination with other grains. Recently, there has been evidence that some coeliac patients may not be able to tolerate oats [Lundin *et al.* 2003]. Additionally, avenin-reactive T-cell lines have been generated *in vitro* from some coeliac patients [Arentz-Hansen *et al.* 2004]. In patients who fail to respond histologically to the gluten-free diet despite good dietary compliance, oats intolerance should be considered [Arentz-Hansen *et al.* 2004]. Ordinarily, patients respond well to a gluten-free diet with a return to normal serology found after 6-12 months [Midhagen *et al.* 2004], while histological recovery should occur within 1-2 years, though patients can still show incomplete recovery at four years [Grefte *et al.* 1988].

As well as the problem of inadvertent consumption of gluten from poorly labelled processed foods, a number of gluten-free food products on the market contain trace amounts of contaminating gluten. Thus a safety threshold for daily gluten consumption is required. The Codex Alimetarius Commission currently recommends a limit of 200ppm for gluten-free food, although this threshold was set in 1983 and requires reconsideration [Stern *et al.* 2001]. A recent study to determine a reasonable limit for residual gluten recommended the threshold to be set at 100ppm, provided the daily flour intake is less than or equal to 300g which would allow for a daily maximum of 30 mg of gluten [Collin *et al.* 2004]. However, not all coeliac patients have the same sensitivity to gluten, and recently a case was reported where consumption of 1mg of

gluten daily over a period of two years prevented mucosal recovery [Biagi *et al.* 2004].

It is sometimes necessary for newly diagnosed coeliac patients to take additional vitamins and minerals until the intestinal mucosa recovers sufficiently [Kennedy and Feighery 2000]. Patients should receive iron or folate supplementation if a deficiency in these minerals was diagnosed [Green and Jabri 2003].

The gluten-free diet (GFD) is a safe and efficient treatment but adherence to the diet can be difficult and has been demonstrated to constitute a significant social burden [Cerf-Bensussan *et al.* 2003; Pietzak 2005]. Alternative therapies are desirable though high standards of efficiency and safety would have to be demonstrated if the GFD is to be replaced [Cerf-Bensussan *et al.* 2003]. Future research ought to be directed at finding alternatives to the GFD, which will, in turn, increase compliance with treatment. These future potential treatments include the development of genetically detoxified grains, oral and intranasal "coeliac vaccines" to induce tolerance, inhibitors of the effects of zonulin on intestinal permeability, inhibitors of TGase 2, and detoxification of immunogenic gliadin peptides via oral peptidase supplement therapy [Pietzak 2005].

1.2.5 Epidemiology

Previously, coeliac disease was considered a disease occurring mainly in infancy; however, with changing practices of the infant diet and the introduction of wheat at a later stage, the clinical symptoms have become more subtle and diagnosis is now delayed to later childhood and adulthood [Maki *et al.* 1988; Murray *et al.* 2001; Fasano and Catassi 2001]. In adults, peak incidence has been shown to occur in the fifth decade [Feighery 1999].

The prevalence rate of a disease depends on its definition; thus, in coeliac disease, reported prevalence rates may describe only the active, diagnosed cases or may describe the latent (preclinical), silent (undiagnosed) and active (diagnosed) cases. Although reports on the prevalence rate of active, diagnosed cases vary between populations, estimates of the combined latent, silent and active cases are very similar,

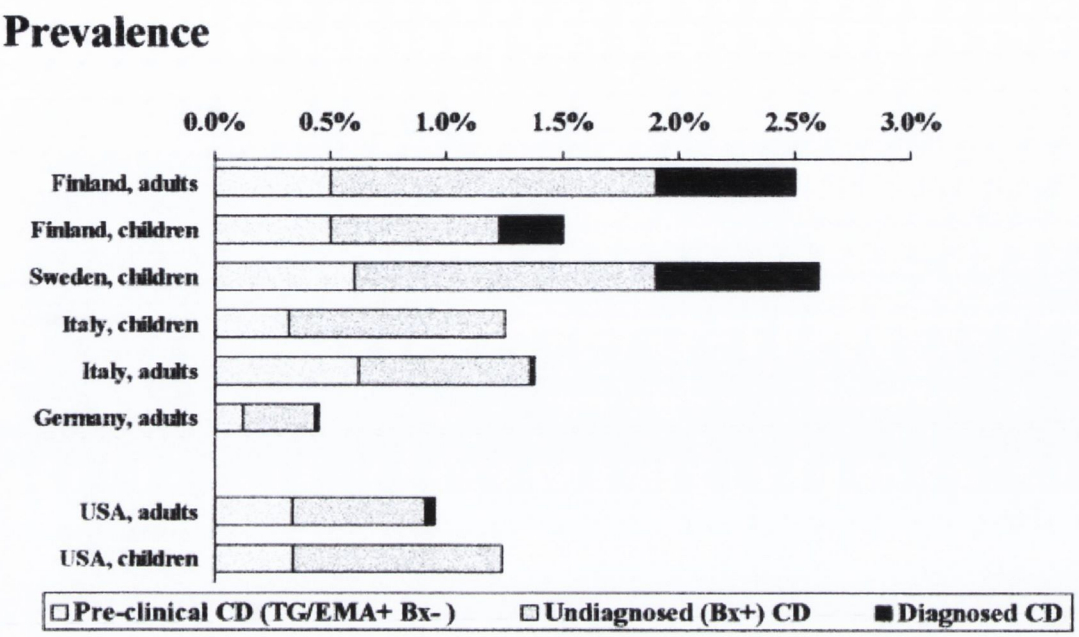


Figure 1.2.1: Prevalence of pre-clinical (latent), undiagnosed (silent) and diagnosed coeliac disease. (from Rewers 2005).

between 0.7-2.0% in most populations (Fig. 1.2.1). Estimates based on seroepidemiologic studies suggest that for each diagnosed case of coeliac disease, there are 3-7 undiagnosed cases and that 1-3% of the general population in Europe and the US will become affected at some point in life [Rewers 2005].

The prevalence of childhood coeliac disease has been reported to be between 0.35% and 1.16% (biopsy proven) in Sweden [Cavell *et al.* 1992; Carlsson *et al.* 2001], between 1% (biopsy proven) and 1.5% (HLA, anti-EMA, anti-TGase 2) in Finland [Maki *et al.* 2003], between 0.44% (biopsy proven) and 0.94% (biopsy proven) in Italy [Catassi *et al.* 1995; Tommasini *et al.* 2004], and between 1% (biopsy proven) and 4% (IgA anti-TGase 2) in the US [Hoffenberg *et al.* 2003]. Other non-European Caucasian populations such as Israel, Argentina, New Zealand and Australia have reported similar rates [Shamir *et al.* 2002; Gomez *et al.* 2001; Cook *et al.* 2000; Hovell *et al.* 2001].

The recent increase in the incidence rates of coeliac disease is most likely due to the increased use of serological screening which leads to increased diagnoses of milder cases [Murray *et al.* 2003].

1.2.6 Complications of coeliac disease

Refractory coeliac disease (RCD) is a form of coeliac disease which is either non-responsive to gluten-free diet or after an initial period of improvement reverts to non-responsiveness. Recent evidence indicates that RCD is a low-grade intraepithelial lymphoma, characterized by massive expansion of IELs with normal cytology but with clonal TCR γ rearrangements and an aberrant phenotype containing intracellular CD3ε (iCD3ε+) but lacking surface expression of CD3-TCR complexes (sCD3ε) [Cellier *et al.* 1998; Verkarre *et al.*. 2003]. The malignant nature of IELs in RCD is supported by frequent association with ulcerative jejunitis, a previously described premalignant condition [Ashton-Key *et al.* 1997], dissemination of clonal IEL to the entire gastrointestinal epithelium [1.232], and subsequent development of Enteropathy-associated T-cell Lymphoma (EATL) sharing the same clonal profile [Charbonnel *et al.* 1998; Daum *et al.* 2000].

Enteropathy-associated T-cell Lymphoma (EATL) is the most common primary gastrointestinal T-cell lymphoma in coeliac disease and the most serious complication of the disease. It has a peak incidence in the sixth decade of life, indicating that many years of latency need to occur between the onset of coeliac disease and the development of EATL [Catassi *et al.* 2005]. The neoplastic cell of EATL is most commonly $CD3^+$, $CD4^-$, $CD8^-$, $CD103^+$ and contains cytotoxic granules recognized by the TIA-1 (T-cell intracellular antigen) antibody [Bagdi *et al.* 1999]. EATL commonly develops in the jejunum but may also be found in the ileum and lymph nodes and less frequently in the stomach and the colon. It is often multifocal with ulcerative lesions Because EATL is often disseminated at diagnosis, extraintestinal presentations are not uncommon in the liver, spleen, thyroid, skin, nasal sinus, and brain [Catassi *et al.* 2005]. The prognosis of the disease is poor, although it may improve with chemotherapy and surgery. In a recent study of EATL patients, survival at 30 months was only 13% [Howdle *et al.* 2003].

Nutritional complications are common in coeliac disease and result from malabsorption. Weight loss, osteoporosis and iron-deficiency anaemia are the most frequent presenting conditions, though deficiencies of calcium, magnesium, zinc, copper, selenium, thiamin, niacin, pyridoxine, folic acid, vitamin B12 and vitamins A,D,E,K have been reported [Kennedy and Feighery 2000].

1.2.7 Associated disorders

Dermatitis herpetiformis (DH) is a cutaneous manifestation of coeliac disease, identified by the presence of granular IgA in dermal papillae. Patients develop intensely pruritic papulovesicles and excoriations on the elbows, knees, buttocks and scalp [Zone 2005]. In 1966, the link between coeliac disease and DH was discovered and it was shown that DH could also be cured by means of a gluten-free diet [Marks *et al.* 1966]. DH patients have been shown to have an enteropathy of the gut ranging from a type 1 to type 3c lesion, with 20% of cases having complete flattening of the villi [Zone 2005]. DH is a relatively rare disorder with the prevalence reported as 0.04% in Sweden [Moi 1984] and 0.001% in the state of Utah, USA [Smith *et al.* 1992]. It is generally not found in young children and mean age of onset has been reported as 40.1 years for men and 36.2 years for women [Zone 2005], though a recent

case was reported of an eight-month old infant presenting with DH [Lemberg *et al.* 2005].

Other skin disorders reported to reverse with gluten-free diet are alopecia areata, psoriasis, and aphthous stomatitis [Jokinen *et al.* 1998; Fessatou *et al.* 2003; Michaelsson *et al.* 2000].

Gluten ataxia is described as immunologic damage to cerebellum, posterior spinal columns, and peripheral nerves in gluten-sensitive individuals. The term gluten ataxia was first proposed by Hadjivassiliou *et al.* in 1998, who showed that 41% of patients with idiopathic ataxia had anti-gliadin antibodies [Hadjivassiliou *et al.* 1998]. The disease is characterised by ataxia (predominantly gait ataxia), the presence of anti-gliadin antibodies, the HLA haplotype associated with gluten sensitivity (72% reported to be DQ2), the presence of anti-Purkinje cell antibodies, the presence of high levels of interferon-γ-inducible chemokine CXCL10, often oligoclonal bands in the cerebrospinal fluid, and the presence of inflammatory pathology of the cerebellum at post-mortem [Hadjivassiliou *et al.* 2003a]. Evidence of a clinical response to the gluten-free diet, in the form of improvement of neurological tests and subjective clinical impressions, has been reported [Hadjivassiliou *et al.* 2003b]. The pathogenesis of the disease remains to be determined; however, it has been shown that antibody cross-reactivity between antigenic epitopes on Purkinje cells and gluten peptides exists [Hadjivassiliou *et al.* 2002].

Other neurological syndromes reported to be associated with coeliac disease are epilepsy, myoclonus, internuclear ophthalmoplegia, multifocal leucoencephalopathy, dementia and peripheral neuropathies [Wills 2000].

An association between coeliac disease and type 1 diabetes has long been recognised with the prevalence of coeliac disease in type 1 diabetic patients being between 3% and 6% [Dube *et al.* 2005]. It has been suggested that coeliac disease may precede the development of Type 1 diabetes, with studies showing that patients in whom coeliac disease was identified and treated in early childhood had a lower rate of developing diabetes than children in whom coeliac disease was diagnosed later in childhood or as

adults [Ventura *et al.* 1999]. Auto-antibodies against islet cells are frequently present in untreated coeliac disease but disappear with introduction of a gluten-free diet [Ventura *et al.* 2000].

Other autoimmune conditions associated with coeliac disease are Crohn's disease, Hashimoto's thyroiditis, Grave's Disease, autoimmune hepatitis and connective tissue diseases such as rheumatoid arthritis, systemic lupus erythematosus and Sjögren's disease [Ventura *et al.* 1999].

1.3 Pathogenesis of Coeliac Disease

1.3.1 Aetiological factors

1.3.1.1 Genetics

Coeliac disease clusters in families with a sibling relative risk (λ_S) of 20-60 [Bevan *et al.* 1999] and a high concordance rate of 75% between monozygotic twins [Greco *et al.* 2002], indicating a strong genetic component to this disease.

MHC class II molecules are encoded in humans in the regions HLA-DQ, -DR and –DP (section 1.1.2.1). Approximately 95% of coeliac patients express HLA-DQ2, while the remaining patients generally express HLA-DQ8. The DQ2 molecule can be encoded by the DR3-DQ2 haplotype giving DQA1*0501, DQB1*0201 (termed DQ2.5 by Vader *et al.* in 2003) or by DR7-DQ2 haplotype giving DQA1*0201, DQB1*0202 (termed DQ2.2 by Vader *et al*) [Vader *et al.* 2003a]. The DQ2 haplotype on its own does not necessarily predispose for coeliac disease but a gene dose effect is evident for the ability of DQ2.5 to predispose [Vader *et al.* 2003a]. A homozygous expression of DQ2.5 gives a 100% risk of developing coeliac disease, while a heterozygous expression of DQ2.5/DQ2.2 gives a 50% risk of developing the disease (trans-dimers A1*0501, B1*0201 and A1*0501, B1*0202 predispose while cis-dimers A1*0201, B1*0202 and A1*0201, B1*0201 do not). The DR5-DQ7 haplotype, DQA1*0505, DQB1*0301 when heterozygously encoded in trans with either DR3 or DR7 can also result in expression of DQA1*0505, DQB1*0202 which predisposes for coeliac disease [Sollid 2002].

The DQ8 molecule, encoded by DR4-DQ8 giving DQA1*0301,DQB1*0302 has been less studied though it is generally considered that DQ8 is a predisposing haplotype, a hypothesis supported by functional data [Cerf-Bensussan *et al.* 2003].

1.3.1.2 Tissue transglutaminase

Tissue transglutaminase (TGase 2) belongs to the transglutaminase family of enzymes which catalyse the post-translational modification of proteins in a calcium-dependent manner. TGase 2 is found in many different tissue types and can be expressed both intracellularly and extracellularly [Reif and Lerner 2003]. Intracellularly, TGase 2 is

involved in the regulation of several biological processes including cellular proliferation, differentiation and apoptosis [Griffin *et al.* 2002]. Extracellularly, the functions of TGase 2 include extracellular matrix assembly, cell adhesion and wound healing [Aeschilmann *et al.* 1994; Patel *et al.* 1985].

TGase 2 catalyses the cross-linking of proteins, connecting a glutamine donor with a glutamine acceptor resulting in the formation of an isopeptidyl bond. This is known as transamidation [Freitag *et al.* 2004]. In the absence of an appropriate glutamine acceptor, TGase 2 can deamidate certain glutamine residues of donor proteins [Freitag *et al.* 2004]. The ratio of deamidation to transamidation in the presence of primary amines is markedly increased when pH is less than 7.3, which is the case in the small intestine (pH 6.6) [Fleckenstein *et al.* 2002]. Gliadin has been shown to act as a donor substrate for TGase 2, with glutamine residues being deamidated to glutamic acid, creating a negative charge on the protein [Caputo *et al.* 2004].

Transglutaminases are involved in the pathogenesis of many diseases through their cross-linking function [Kim *et al.* 2002]. Deficiency in factor XIIIa, a TGase isoform which crosslinks fibrin monomers, causes impaired clotting [Freitag *et al.* 2004], while increased TGase activity has been linked to formation of senile plaques and neurofibrillary tangles in Alzheimer's disease and intraneuronal aggregates in Huntington's disease [Freitag *et al.* 2004]. TGase 3 has been shown to be involved in the pathogenesis of dermatitis herpetiformis, a related disorder to coeliac disease (section 1.2.7) most likely through the dermal deposition of TGase 3 + IgA anti-TGase 3 antibody complexes [Sárdy *et al.* 2002]. Studies on active coeliac disease revealed that TGase 2 is expressed at the epithelial brush border as well as being expressed extracellularly in the subepithelial region [Molberg *et al.* 1998].

TGase 2 was first described as the autoantigen in coeliac disease in 1997 by Dieterich *et al.* [1997]. Antibodies to TGase 2 are observed in coeliac patients consuming gluten and have been used diagnostically as a very specific marker for the disease [Dieterich *et al.* 1998; Troncone *et al.* 1999]. These antibodies are thought to occur when TGase 2 crosslinks to gliadin, acting as a hapten to generate autoantibodies [Caputo *et al.* 2004]. Whether anti-TGase 2 autoantibodies have a role in the pathogenesis is still

controversial. Anti-TGase 2 antibodies from coeliac patients were found to inhibit fibroblast-induced differentiation of epithelial cells *in vitro*, suggesting a role for TGase 2 in the mucosal remodelling seen in coeliac disease[Haltunnen *et al.* 1999]. TGase 2 has been shown to be necessary for activation of TGF-β which is also involved in the differentiation of intestinal epithelium [Kurokawa *et al.* 1987]. TGF-β suppresses T-cell activity; hence, decreased TGF-β could lead to increased activation of T_H1 cells producing inflammatory cytokines [Letterio *et al.* 1998; Hansson *et al.* 2002]. An inhibitory effect (up to 39% inhibition) of anti-TGase 2 antibodies on TGase activity has been demonstrated by Eposito *et al.* [2002].

1.3.1.3 Gliadin

Cereal grains contain about 7-16% protein depending on the genotype and growing conditions. Three types of cereal protein are known: structural, metabolic and storage proteins. Storage proteins make up 70-80% of total grain protein and occur exclusively in starchy endosperm. Traditionally, cereal proteins have been grouped into four classes according to their solubility. These are albumins, globulins, prolamins and glutelins. Prolamins are monomeric storage proteins and glutelins are aggregated storage proteins. Prolamins are the alcohol-soluble protein fraction of the cereal that is responsible for toxicity in coeliac disease, and which in wheat is termed gliadin. The term "prolamin" reflects the high content of proline and amide nitrogen (now known to be derived from glutamine) of which all types of prolamin are made up, though the properties can vary. The main function of prolamins is storage of nitrogen, sulphur and carbon, which is broken down into energy during seed germination.

The amino acid composition of the prolamins displays an association with the phylogeny of the cereals (Fig.1.3.1). This is reflected in the toxicity of different cereals. Prolamins of cereals in the genus hordeae (also known as triticeae), such as wheat, barley and rye have high contents of glutamine and proline (35-37mol-%, and 17-23mol-% respectively). Other cereals, considered "safe" for coeliac patients, such as rice, millet and maize have prolamins which are much lower in glutamine and proline content, but rich in alanine (9-14mol-%) and leucine (12-19mol-%). Oats lie in between with glutamine levels as found in the *hordeae* family and levels of proline and leucine similar to the safer cereals.

While it has been known since 1950 [Dicke] that gliadin is toxic to coeliac patients, the exact part of this protein which confers its damaging properties remains to be clarified. Van de Kamer and Weijers [1955] noted that the high glutamine content of gliadin contributed to its toxicity while Krainick and Mohn [1959] observed that both glutamine and proline were important component amino acids in toxic peptides. Frazer *et al.* [1959] demonstrated that a soluble extract resulting from a peptic-tryptic digest of gluten- denoted as Frazer's Fraction III- remained toxic to coeliac patients [Frazer *et al.* 1959]. In 1970, Hekkens *et al.* prepared an α-gliadin fraction and demonstrated that it was toxic to coeliac patients [Hekkens *et al.* 1970; 1978]. Subsequently, the remaining gliadin fractions β-, γ- and ώ-gliadin have all been shown to be toxic [Jos *et al.* 1982; Wieser *et al.* 1982]. With the publication of the complete amino acid sequence of A-gliadin, an aggregable α-type gliadin by Kasarda *et al.* in 1984, specific peptides of known sequence could be analysed for toxicity [Kasarda *et al.* 1984].

Weiser *et al.* were the first group to isolate a toxic peptide, which they further hydrolyzed into two small, yet remaining active peptides, termed CT-1 and CT-2 [Weiser *et al.* 1984; 1986]. Since then a number of peptides have been shown to have a toxic effect on coeliac tissue. Studies have measured morphometric and immunological responses in a number of experimental systems, most notably through organ culture and more recently through the use of intestinal T-cell lines/clones. A summary of the discovered gliadin epitopes is given in table 1.3.1. Initial studies mainly found toxic sequences in the first portion of α-gliadin, with the peptide α: 31-49 or similar derivatives commonly proving toxic [de Ritis *et al.* 1988; Sturgess *et al.* 1994; Shidrawi *et al.* 1995; Maiuri *et al.* 1996]. Only one early study discovered a toxic sequence in γ-gliadin [Fluge *et al.* 1994]. In 1998, it was discovered that TGase 2 selectively modifies gliadin peptides by deamidating certain glutamine (Q) residues to glutamic acid (E) thus creating negative charges which aid in binding to HLA molecules [Molberg *et al.* 1998; Van de Waal *et al.* 1998]. Since then, a vast number of epitopes within α- and γ-gliadin have been discovered which in most cases require prior deamidation, although the focus has been placed on epitopes within the 51-89 fraction of α-gliadin [Arentz-Hansen *et al.* 2000; Anderson *et al.* 2000; Arentz-Hansen *et al.* 2002; Shan *et al.* 2002; Martucci *et al.* 2003]. An algorithm was designed to predict epitopes within gluten which described the specificity of deamidation based on

preferential spacing between glutamine and proline [Vader *et al.* 2002a]. This algorithm confirmed the previously described stimulatory α-gliadin peptide 57-68 as well as finding many more stimulatory peptides.

The diversity of gliadin epitopes is greater in paediatric cases than adult with a heterogeneous response to different sets of peptides observed amongst the paediatrics [Vader *et al.* 2002b]. Furthermore, the previously described stimulatory peptides found to create a response in adults cases were found to only create a response in half the paediatric cases, while deamidation was not a prerequisite for recognition of peptides in the paediatric cases. These observations suggest that the initial immune response in a coeliac patient is to a number of gluten peptides and over time cross-reactivity may lead to the development of a diverse and spreading T-cell response with the later response against just a few dominant epitopes with strong DQ-binding capacity [Vader *et al.* 2002b].

Recently, a number of high-molecular-weight glutelin (termed glutenin in wheat) peptides have been found to be stimulatory [van de Wal *et al.* 1999; Vader *et al.* 2002b; Molberg *et al.* 2003], thus changing the opinion that only the prolamin proteins are harmful to coeliac patients. As the high-molecular-weight glutenins are important to the bread-making properties of wheat, this discovery adds further complexity to the challenge of removing the toxic sequences to create a safe form of wheat for coeliac patients.

Table 1.3.1: Toxic and stimulatory peptides of wheat gliadin

Sequence	Designation	Position	Test used	Reference
VPVPQLQPQNPSQQQPQEQVPL	CT-1	α: 3-24	Organ culture	Weiser et al (1986)
VQQQQFPGQQQPFPPQQPYPQPQPFPSQQPY	CT-2	α: 25-55	Organ culture	Weiser et al (1986)
VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF	XT(1-30)	α: 1-30	Organ culture	De Ritis et al (1988)
LGQQQPFPPQQPYPQPQPFPSQQPY	XT(31-55)	α: 31-55	Organ culture	De Ritis et al (1988)
LGQGSFEPSQQN	none given	α: 206-217	Direct installation into duodenum	Mantzaris et al (1991)
RPQQPYPQPQPQ	Peptide V from fraction 9	α: 75-86	Foetal chick intestine, rat liver lysosomes	Cornell et al (1993)
-QIVFPSGQVQWPQQQQPFP	N-terminal of peptide FrIII-2-VIII	FrIII-2-VIII: 1-20 Homologous w/ N-terminal γ-gliadin	Organ culture	Fluge et al (1994)
LGQQQPFPPQQPYPQPQPF	Peptide A	α: 31-49	In vivo	Sturgess et al (1994)
			Organ culture	Shidrawi et al (1995)
LGQQQPFPPQQPY	none given	α: 31-43	Organ culture	Maiuri et al (1996)
PQPQPFPSQQPY	none given	α: 44-55	Organ culture	Maiuri et al (1996)
QQPQQQYPSGQGSFQPSQQNPQAQG	none given	α/β: 198-222	T-cell lines/clones	Van de Waal et al (1998)
QQLPQPQQPQQSFPQQQRPF	γ-1 (134-153)	γ: 134-153(Q148E)	T-cell lines/clones	Sjöström et al (1998)

Sequence	Designation	Position	Test used	Reference
QLQPFPQPQLPY	α-9 (57-68)	α: 57-68 (Q65E)	T-cell clones	Arentz-Hansen et al (2000)
PQPQLPYPQPQLPY	α-2 (62-75)	α: 62-75 (Q65E)	T-cell clones	Arentz-Hansen et al (2000)
QLQPFPQPQLPYPQ	none given	α: 57-73 (Q65E)	Peripheral blood lymphocytes	Anderson et al (2000)
$XXXQ_4XP_6Q_7XP_9(Y,F,W,I,L)_{10}$	Algorithm to predict peptides susceptible to TGase2 deamidation		T cell lines	Vader et al (2002a)
PFRPQQPYPQPQPQ	Glia- α20 (93-106)	α: 93-106	Paediatric T-cell clones	Vader et al (2002b)
VQGQGIIQPQQPAQL	Glia- γ30 (222-236)	γ: 222-236	Paediatric T-cell clones	Vader et al (2002b)
QPQPFPQQSEQSQQPFQPQPF	Glu-21	not known	Paediatric T-cell clones	Vader et al (2002b)
QQXSQPQXPQQQQXPQQPQQF	Glu-5	not known	Paediatric T-cell clones	Vader et al (2002b)
PYPQPQLPY	DQ2-α-III	α-2: 65-75 (Q72E)	T-cell lines/clones	Arentz-Hansen et al (2002)
FPQQPQQPYPQQP	DQ2-γ-III	γ-5: 66-78 (Q68E, Q71E)	T-cell lines/clones	Arentz-Hansen et al (2002)
FSQPQQQFPQPQ	DQ-γ-IV	γ-5: 102-113 (Q106E, Q108E)	T-cell lines/clones	Arentz-Hansen et al (2002)
LQPQQPFPQQPQQPYPQQPQ	DQ-γ-V	γ-5: 60-79	T-cell lines/clones	Arentz-Hansen et al (2002)
LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF	33-mer	α-2: 57-89	T-cell lines	Shan et al (2002)
SQQPYLQLQPFPQPQLPYSQ	A-gliadin 51-70	α: 51-70	Organ culture	Martucci et al (2003)

1.3.2 Pathomechanisms

The immune response to gliadin has been studied in detail and much insight into the pathogenesis of coeliac disease has been provided. An interplay between adaptive immunity and innate immunity is now considered to occur, though to what extent each part plays is still controversial.

1.3.2.1 Adaptive response

The adaptive immune response is characterised by a specific and memory Th1 reaction to gliadin peptides. The first step is the deamidation of gliadin peptides by TGase2, creating negative charges on the peptides that allow them to bind to the DQ2 or DQ8 peptide groove of the antigen-presenting cells. These modified peptides are then presented to $CD4^+$ T-cells in the lamina propria which become activated. The T-cells, upon activation, release pro-inflammatory cytokines in particular IFN-γ which can be directly toxic to epithelial cells or can activate macrophages to release damaging components such as matrix metalloproteinases [Cerf-Bensussan *et al.* 2003]. IFN-γ regulates the expression of a key T-box transcription factor, T-bet which in turn controls IFN-γ production in an autocrine positive feedback loop. This mechanism is dependent on signal transducer and activator of transcription 1 (STAT1) and independent of the Th1 polarising cytokine, IL-12 [Monteleone *et al.* 2004].

This model of pathogenesis has been well accepted; however, what is not clear is how the gliadin peptides breach the epithelial barrier and what the initial trigger in the sequence of events is.

1.3.2.2 Permeability

Due to its high proline content, gliadin is unusually resistant to digestion by intraluminal and brush border enzymes [Shan *et al.* 2002]. The pathways by which the partially digested gliadin peptides cross the epithelial barrier and gain access to the subepithelial region are not clear. Under normal circumstances, up to 90% of proteins cross the epithelial barrier through the transcellular pathway where lysosomal degradation occurs and proteins are converted into smaller non-immunogenic peptides. The other portion of proteins cross the epithelial barrier by the passive, paracellular pathway in intact form, resulting in antigen-specific immune responses. Peptides may

be taken across the mucosa by dendritic cell processes that can cross the epithelial layer in a manner similar to that proposed for bacteria [Rescigno *et al.* 2001].

One possible setting for increased uptake of gliadin peptides is during transient infection, where activation of dendritic cells, macrophages and other immune cells in the lamina propria leads to increased production of pro-inflammatory cytokines such as IFN-γ. These cytokines can increase epithelial permeability, enhance further pro-inflammatory cytokine production, up-regulate the expression of HLA molecules and facilitate the activation of additional immune and inflammatory cells, all of which could lead to a pathologic response to gliadin peptides. Evidence supporting this model is seen from treatment with IFN-α, for chronic hepatitis, which has been shown to induce the onset of coeliac disease [Bardella *et al.* 1999]. At the same time, tissue repair associated with infection causes release of TGase2 which would increase the number of modified peptides capable of binding to HLA DQ2/8 on activated APCs.

Another potential route for gliadin peptides to breach the epithelial layer is through increased paracellular transport. Increased permeability through tight junctions which modulate paracellular passage, has been demonstrated in coeliac disease [Cooper *et al.* 1983; Bjarnason *et al.* 1983; Schulke *et al.* 1998]. Fassano *et al.* [2000] discovered a toxin to the tight junction protein zonula occludens, which they termed zonulin, and demonstrated increased expression in the submucosa of coeliac intestinal tissue. This group also showed that gliadin can induce zonulin release from intestinal epithelial cells *in vitro* [Clemente *et al.* 2003]. Zonulin binds to its surface receptor, inducing a protein kinase C- mediated polymerisation of intracellular actin filaments which are directly connected to structural proteins of the tight junction, thus regulating epithelial permeability [Wang *et al.* 2000]. One argument against a major paracellular leakage of gliadin peptides through the mucosa of coeliac patients comes from studies measuring the amount of various peptides to be transported across the epithelium. It was demonstrated that a significant increase in peptide 31-49 fluxes occurred in untreated coeliac biopsy specimens compared to controls while no increase in peptide 57-68 or peptide 56-89 fluxes was observed [Matysiak-Budnik *et al.* 2003]. This would not have been the case if a non-specific leakage via the paracellular route was occurring and points instead to a transcellular route of absorption for gliadin peptides.

1.3.2.3 Innate response

Recent data has suggested that gliadin can stimulate an innate response from the immune system with IL-15 likely to play a central role. The peptide 31-49 (or shorter peptide 31-43) has been shown to be toxic to coeliac intestinal tissue with a decrease in mean enterocyte height [Shidrawi *et al.* 1995; Maiuri *et al.* 1996]. In another study, the p31-43 peptide was shown to induce ICAM-1 and HLA-DR mRNA in coeliac biopsies within 3 hours of culture and thus before T-cell activation would have occurred [Maiuri *et al.* 2003]. Mauiri *et al.* [2003] also demonstrated that p31-43 activates the intestinal macrophages in coeliac tissue, inducing macrophage production of IL-15, their expression of COX-2, and the phosphorylation of p38 MAP kinase. This study showed that p31-43 caused activation and maturation of cells belonging to the innate immune system but not T-cells, while a recent study showed p31-49 could up-regulate the innate surface molecule MICA on epithelial cells of coeliac biopsies, a process which could be blocked by anti-IL-15 [Hüe *et al.* 2004]. Furthermore, the toxic peptide was found to induce intraepithelial migration of T-cells.

One of the hallmarks of coeliac disease is the huge increase in IELs, a feature not seen in other inflammatory conditions of the gut with lamina propria T-cell activation such as Crohn's disease and autoimmune enteropathy [Jabri *et al.* 2000]. There are two subsets of IELs increased in coeliac disease; however, unlike the TCR-$\alpha\beta^+$ IELs which are only increased in untreated coeliac disease, the TCR-$\gamma\delta^+$ IELs are present in increased amounts in latent coeliac disease and remain increased several years after initiation of a gluten-free diet [Kutlu *et al.* 1993; Iltanen *et al.* 1999]. The function of TCR-$\gamma\delta^+$ IELs is less understood but it is believed they act as sentinels allowing immediate response to epithelial damage, thus belonging to innate immunity. It is thought the persistent increase in TCR-$\gamma\delta^+$ IELs after removal of gluten from the diet may be caused by the long-lasting overexpression of IL-15 in the intestinal epithelium [Mention *et al.* 2003]. Organ culture studies showing that IL-15 rapidly increases the number of TCR-$\gamma\delta^+$ IELs in treated coeliac biopsies support this hypothesis [Maiuri *et al.* 2001].

Exposure to IL-15 up-regulates the natural killer group 2, member D (NKG2D) receptor on activated cytotoxic T-lymphocytes which, signaling through the adaptor

DAP10, can lyse target cells by interacting with their stress-induced ligand MICA, also shown to be up-regulated by IL-15 [Meresse *et al.* 2004; Hüe *et al.* 2004]. Thus, the high levels of IL-15 and the constant exposure to antigen in the intestine of untreated coeliac patients allows the IELs to lyse the epithelial cells independently of further engagement of the TCR. This may explain why in refractory coeliac disease, where gluten is no longer present, the intestinal lesion fails to heal. Studies have shown that refractory coeliac disease and EATL are two stages of a malignant process arising from the IEL compartment [Cellier *et al.* 1998; 2000]. IL-15 promotes the survival and growth of abnormal clonal IELs in *ex vivo* studies and might therefore support the emergence of lymphoma [Meresse *et al.* 2004].

The evidence points to a defect in the regulation of IL-15 in coeliac patients. Uncovering such a defect, however, will first require deciphering of the complex mechanisms which control IL-15 translation and expression.

Chapter 2

Analysis of the inflammatory response to barley and rye prolamins in coeliac mucosa following *ex vivo* culture.

2.1 Introduction

The treatment for coeliac disease is a life-long gluten-free diet. The current standard for a gluten-free diet, adopted by the Codex Alimentarius Commission in 1976 (amended in 1983, reviewed in 2003 and presently awaiting redefinition), classified gluten as those storage proteins commonly found in the cereals wheat, oats, rye, barley and triticale (wheat/rye hybrid) [Codex Alimentarius 2003]. Research into the toxic properties of wheat has been extensive while in recent years oats have received much attention due to opposing reports (section 1.2.4). To date, barley and rye have received little attention and a lack of scientific evidence for their presumed toxicity exists.

Shortly after the damaging effect of wheat in coeliac patients was first discovered [Dicke 1950], rye and oats were also implicated and shown to cause increased faecal fat excretion in coeliac patients [Dicke *et al.* 1953]. Barley was not investigated until 1962 when Rubin *et al.* instilled slurries of wheat, barley, rye or oats into the ileum of treated coeliac patients [Rubin *et al.* 1962]. After the instillations, they noted typical clinical symptoms and histological abnormalities in the biopsies taken from patients challenged with wheat, barley and rye but not oats. There have been few experiments since, examining the local, small intestinal effect of barley and rye in coeliac patients. An early oral challenge study showed that barley ingestion resulted in reduced D-xylose excretion in six out of ten patients [Baker and Read 1976]. However, the D-xylose test has been shown to be unreliable and lacking in specificity when detecting patients with mucosal damage compared to patients with normal jejunal mucosa [Sladen and Kumar 1973; Krawitt and Beeken 1975]. Another oral challenge study examined the effect of barley and rye on five and two treated coeliac patients respectively: a decrease in disaccharidase enzyme levels in the jejunal biopsies of all patients was found, as was a reduction in surface to volume ratio in the jejunal biopsies of six of the seven coeliac patients [Anand *et al.* 1978]. Neither of these studies [Baker and Read 1976; Anand *et al.* 1978] was carried out on control patients and in both cases the duration of the challenge period was just one month.

More recently, with the availability of T-cell lines and clones to test antigenic peptides and algorithms to predict T-cell stimulatory sequences, barley and rye have been

shown to contain many potentially immunogenic peptides [Vader *et al.* 2002a]. Two peptides of both barley and rye, with sequences homologous to known stimulatory wheat peptides, have been shown to cause an immune response in T-cell lines and/or clones [Vader *et al.* 2003b]. Such lines and clones are useful for performing large numbers of experiments and readily demonstrate the T-cell response to stimuli. However, they do not necessarily reflect the small intestine *in vivo,* where a heterogeneous population of cells interact in response to a stimulus. The organ culture system, using whole tissue, may mirror the *in vivo* situation more accurately and previous studies have shown gliadin to elicit an immunogenic response in coeliac biopsies [Nilsen *et al.* 1998; Kilmartin *et al.* 2003].

The aim of this study was to develop an *ex vivo* system to assess the inflammatory response of coeliac intestinal mucosa to barley and rye. It has been reported that cytokine expression is a quantifiable marker of an immune response and the pro-inflammatory cytokines interferon γ (IFN-γ) and interleukin 2 (IL-2) have been shown to be over-expressed in response to wheat gliadin in organ cultures of coeliac biopsies [Nilsen *et al.* 1998]. Thus, duodenal biopsies from coeliac patients and controls were cultured in the presence of wheat, barley and rye prolamins and the responses were compared. IFN-γ and IL-2 mRNA and protein expression were quantified by TaqMan RT-PCR and ELISA respectively.

2.2 Materials and Methods

2.2.1 Patients

Eight duodenal biopsy specimens were obtained from 22 patients with coeliac disease and 23 control subjects via oesophago-gastro-duodenoscopy. Clinical details are outlined in table 2.2.1. The control group consisted of patients undergoing endoscopy for investigation of upper gastrointestinal symptoms. Ethics approval for this study was granted by the Joint Ethics Committee of St. James's and Tallaght Hospitals, Dublin, Ireland.

2.2.2 Prolamin fractions

The prolamin preparations were a gift from Herbert Weiser and were produced as follows. Kernels of the German wheat cultivar Rektor, rye cultivar Danko and barley cultivar Aura were milled to flour with an ash content of 0.55 % using a laboratory mill (Brabender Quadrumat Junior). The flours were defatted with light petroleum (boiling range 40 – 60°C) and air-dried. The defatted flours (50g) were extracted stepwise three-times with 200 ml of a salt solution (0.4 mol/l NaCl + 0.067 mol/l NaK phosphate, pH 7.6) and three-times with 200ml of 60 % (v/v) aqueous ethanol using an Ultra Turrax homogeniser for 5 min at room temperature (RT). The suspensions were centrifuged at 40,000g for 15 min at RT and the supernatants of the three ethanol extracts were combined. These were concentrated to approximately 200ml using a vacuum evaporator at 40°C, dialysed against 0.01mol/l acetic acid until free from chloride and freeze-dried.

The stepwise enzymatic hydrolysis of the prolamins was performed with pepsin (Sigma P3286) and trypsin (Sigma T1763) both attached to agarose according to Bolte *et al.* [1996]. Prolamin (1g) was dissolved in 50ml of diluted HCl (pH 2.0) and 100mg (4000 U) of pepsin was added. The mixtures were magnetically stirred at 37°C for 2 hours then centrifuged at 6,000g for 20 min at RT. The pH of the supernatants was adjusted to 7.8 with 0.1mol/l NaOH and 1ml of the trypsin suspension (25 U) was added. The mixtures were magnetically stirred at 37°C for a further 2 hours and the pH was adjusted to 7.0 with 0.1mol/l HCl. Centrifugation followed for 20 min at 6,000g at RT. The supernatants were then freeze-dried. Control RP-HPLC on C18 silica gel indicated complex peptide patterns for the gliadin, secalin and hordein digests.

Table 2.2.1: Patient Clinical Details

Patient No.	Sex	Age (yrs.)	Gluten-free diet (yrs)	Serum Antibodies	Histology Grade
1	M	59	<1	EMA +	1
2	F	40	<1	EMA +	3c
3	F	37	1	EMA -	2
4	M	68	7	EMA -	3a
5	F	40	<1	tTG -	0
6	M	46	1	tTG -	1
7	F	55	7	NK	0
8	F	48	2	EMA -	0
9	M	52	4	EMA -	3a
10	F	49	1	tTG -	3a
11	F	38	<1	EMA +	3a
12	F	52	2	tTG -	0
13	F	37	7	EMA weak +	1
14	F	43	<1	EMA +	1
15	F	59	<1	NK	1
16	F	61	6	tTG -	1
17	F	61	8	EMA -	0
18	F	46	1	NK	3c
19	M	50	4	NK	0
20	F	55	6	tTG -	2
21	F	33	<1	NK	1
22	F	44	2	NK	0
23	F	45	N/A	EMA -	0
24	M	39	N/A	NK	0
25	M	51	N/A	EMA-	NK
26	F	27	N/A	EMA -	0
27	M	66	N/A	NK	0
28	M	21	N/A	NK	0
29	M	57	N/A	NK	0
30	F	58	N/A	NK	0
31	M	45	N/A	NK	0
32	F	37	N/A	tTG -	NK
33	M	28	N/A	tTG -	0
34	M	34	N/A	tTG -	0
35	F	24	N/A	NK	0
36	F	24	N/A	tTG -	0
37	F	51	N/A	NK	0
38	M	37	N/A	NK	0
39	M	27	N/A	EMA -	0
40	M	58	N/A	NK	0
41	M	31	N/A	EMA -	0
42	F	52	N/A	NK	0
43	F	37	N/A	NK	0
44	M	40	N/A	NK	0
45	F	59	N/A	NK	0

EMA, endomysial antibody; tTG, tissue transglutaminase antibody; NK, not known.Histology grade: 0=normal, 1=raised intraepithelial lymphocytes (IELs), 2= increase in IELs with crypt hyperplasia, 3a= increase in IELs, crypt hyperplasia, mild villous atrophy, 3b= increase in IELs, crypt hyperplasia, marked villous atrophy and 3c= total villous atrophy

2.2.3 Organ Culture

The organ culture system was based on that originally described by Browning and Trier [1969]. Biopsies were orientated villous side up on a nylon mesh gauze (Millipore, Ireland) and placed in the central well of a 2-ml tissue culture dish (Falcon, UK) containing RPMI-1640 (Gibco BRL, UK) supplemented with 15% foetal calf serum (Sigma, USA) and a 1% antibiotic/antimycotic solution (Gibco BRL), such that the biopsies were sitting on the surface of the media. Eight biopsy specimens were obtained from each patient: four were cultured in one dish with RPMI and four cultured in another dish with RPMI plus 5mg/ml PT digest of gliadin, hordein or secalin. Extra media was placed in the outer well of each dish in order to prevent evaporation from the central well. The culture dishes were placed in an organ culture chamber, gassed with a mixture of 95% O_2 5% CO_2, and incubated at 37°C for 4 hours.

2.2.4 RNA extraction

Total RNA was isolated using the guanidium thiocyanate method originally described by Chomczynski and Sacchi [1987]. All steps were carried out on ice. Biopsy specimens were removed from culture, added immediately to 500 µl denaturing solution (4M guanidium thiocyanate, 25mM sodium citrate; pH7, 0.5% N-lauroylsarcosine, 0.1M 2-mercaptoethanol) and homogenized using a tube pestle. Fifty microlitres of 2M sodium acetate, pH 4 was added to the solution and gently mixed. Saturated phenol (500 µl) was carefully pipetted into the reaction tube followed by 200µl of chloroform/isoamyl alcohol (49:1). The mixture was vortexed vigorously then incubated on ice for 15 minutes with regular mixing, and centrifuged at 10,000g for 15 minutes at 4°C. The upper, aqueous layer was transferred to a fresh tube and 500µl of ice-cold isopropanol was added. The mixture was incubated overnight at -80°C, followed by centrifugation at 10,000g for 20 minutes at 4°C. The supernatant was poured off and the remaining pellet was dissolved in 200µl of denaturing solution. Ice-cold isopropanol (200µl) was added, the solution mixed and incubated at -80°C for 30 minutes. The reaction mixture was centrifuged at 10,000g for 10 minutes at 4°C and the supernatant removed. Four hundred microlitres of 75% ethanol was added to dissolve the pellet and incubated on ice for 15 minutes. The solution was then centrifuged at 10,000g for 5 minutes at 4°C. The supernatant was poured off and the

pellet allowed to air-dry before being resuspended in RNase free water and stored at -80°C.

2.2.5 Reverse transcription

Total RNA (7.5µl) was heated at 85°C for 2 min and then incubated on ice for 1 minute. A master mix containing 5mM $MgCl_2$, avian myeloblastosis virus reaction buffer (50mM Tris-HCl pH 8.3, 50mM KCl, 10mM $MgCl_2$, 10mM DTT, 0.5mM spermidine), 1U/µl RNasin® ribonuclease inhibitor, 1U/µl avian myeloblastosis virus reverse transcriptase, 5ng/µl random primers, 1mM deoxynucleotide triphosphates (Promega, USA) and RNase-free water was produced and 22.5µl of this mix was added to each denatured RNA sample. The reaction was incubated at 42°C for 1 hour and the cDNA samples were stored at -80°C.

2.2.6 Primers and Probes

Primers and probes for IFN-γ and IL-2 were designed using the software Primer Express (Applied Biosystems UK), and were based on the published sequences Genbank Accession No J00219 for human IFN-γ and Genbank Accession No K02056 for human IL-2 (table 2.2.2). Primers or probes were designed to span an intron, thus preventing genomic DNA from being amplified. BLAST searches were performed to confirm the primers were specific for the respective cytokines. Pre-developed assay reagents (Applied Biosystems), incorporating primers and probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used. Melting temperatures ™ of the primers were between 50°C and 60°C, while probe Tm was 10°C higher than that of the primers. IFN-γ and IL-2 probes were labelled at the 5' end with the fluorogenic reporter dye FAM and the GAPDH probe was labelled with VIC dye. All three probes were labelled at the 3' end with the quencher dye TAMRA.

Table 2.2.2: Designed primer and probe sequences

Primers and Probes	Sequences	Amplicon size
IFN-γ		96bp
Probe	5' GTCACTCTCCTCTTTCCAATTCTTCAAAATGC 3'	
Forward primer	5' TTCAGATGTAGCGGATAATGGAAC 3'	
Reverse primer	5' GAGACAATTTGGCTCTGCATTATTT 3'	
IL-2		88bp
Probe	5' ATGCTCACATTTAAGTTTTACATGCCCAA 3'	
Forward Primer	5' GAATTAATAATTACAAGAATCCCAAACT 3'	
Reverse Primer	5' AGATGTTTCAGTTCTGTGGCC 3'	

IFN-γ, Interferon-gamma; IL-2, Interleukin 2.

2.2.7 TaqMan PCR

The ABI TaqMan 7700 (Applied Biosystems) was used to perform real-time PCR. Each cDNA sample was tested in triplicate in a 25μl reaction mixture containing 2μl of cDNA, 0.3pmol/μl forward primer, 0.3pmol/μl reverse primer, 0.1pmol/μl probe, and Universal PCR Master Mix (PCR buffer, deoxynucleotide triphosphates, AmpliTaq Gold, uracil-N-glycosylase) (Applied Biosystems). A number of controls were included on each plate: a no template control (NTC), where RNase-free water was added in the place of cDNA in the reaction mix; a non-amplification control (NAC), where SDS was added to prevent DNA amplification, and a positive control, where cDNA from PBMCs (stimulated with PMA/OKT3 for 4 hours) was added. The reaction was carried out using the settings described in appendix II-A.

Emission from released reporter dye correlated to accumulation of PCR product after a number of cycles. Samples were compared across a threshold cycle (Ct) where there is an inverse relationship between the amount of template present and the Ct value. These Ct values were used to calculate relative abundance of mRNA transcripts using the formula $2^{-\Delta\Delta Ct}$

where ΔCt = Ct of the target gene - Ct of the internal control (GAPDH), and

$\Delta\Delta Ct = \Delta$ Ct (prolamin-stimulated sample) - Δ Ct (unstimulated sample)

Results are expressed as a fold-change based on the ratio of cytokine mRNA in cultures stimulated with prolamin to unstimulated cultures.

2.2.8 ELISA

The culture supernatants of biopsy samples were used for protein quantification.

2.2.8.1 IFN-γ ELISA

IFN-γ ELISA was carried out using the human IFN-γ ELISA assay (Mabtech, Sweden). A 96-well ELISA plate was coated with 2μg/ml capture antibody diluted in PBS and incubated overnight at 4°C. The plate was washed three times with PBS/0.05% Tween, blocked with the same solution for 1 hour at RT and washed again. IFN-γ standard, a recombinant human protein (R&D Systems, US), was adjusted to a starting concentration of 5ng/ml (in RPMI media) with serial 1 in 2 dilutions performed until a final standard of 0.078ng/ml was created. These seven concentrations of standard along with undiluted supernatant from each sample were loaded in triplicate onto the plate. A positive control was created prior to the ELISA experiment. Peripheral blood mononuclear cells ($1x10^6$ cells/ml) were cultured in a 96-well plate with 25ng/ml phorbol myristate acetate (PMA) and 30μg/ml anti-CD3 (clone OKT3) for 24hours. Following stimulation, culture supernatant was removed and aliquoted. For each ELISA experiment, this positive control was used neat and at a 1 in 2 dilution. A negative control of RPMI media was also run. The samples, standards and controls were allowed to incubate for 2 hours at RT after which time the plate was washed. Biotinylated anti-IFN-γ antibody (1μg/ml) diluted in PBS/Tween was added and incubated for 1 hour at RT. Unbound antibody was removed by washing as before. Streptavidin-alkaline phosphatase was added to each well at a concentration of 1:1000 in PBS/Tween, allowed to react for 1 hour and unreacted solution washed off. Finally, the substrate solution p-nitro-phenylphosphate (1mg/ml p-nitro-phenylphosphate in 10% diethanol amine buffer containing 0.5 mM $MgCl_2$ pH 9.8) was added to the wells and incubated for approximately 20 min. The spectrophotometrical absorbance was measured at 405nm and the absorbance value obtained for each standard was plotted against concentration using the computer program MultiCalc, creating a standard curve. The concentration of IFN-γ protein in the culture supernatant was determined from the standard curve.

2.2.8.2 IL-2 ELISA

IL-2 ELISA was carried out using the human IL2 DuoSet (R&D Systems, US). A 96-well ELISA plate was coated in 4μg/ml capture antibody, diluted in PBS and allowed

to incubate overnight at RT. The plate was washed three times with PBS/0.05%Tween and blocked with block buffer (1% BSA, 5% sucrose, 0.05% NaN_3 in PBS) for 1 hour at RT. The wash step was repeated. IL-2 standard (provided in kit) was adjusted to a concentration of 1000pg/ml in reagent diluent (0.1% BSA, 0.05% Tween in Tris-buffered saline, pH 7.3). Serial 1 in 2 dilutions were performed until a final standard of 15.625pg/ml was created. The seven standards and undiluted supernatant samples were added to the plate in triplicate, along with the controls described in section 2.2.8.1, and allowed to incubate for 2 hours at RT. Following incubation, the plate was washed and 50ng/ml of biotinylated anti-IL-2 antibody diluted in reagent diluent was added and allowed to incubate for a further 2 hours at RT. Excess antibody was removed by washing and streptavidin-horseradish peroxidase diluted in reagent diluent was added to the wells and allowed to react in the dark for 20 min. The washing step was repeated and tetramethylbenzidine added for a further 20 min in the dark. The reaction was stopped with 1M H_2SO_4 and the spectrophotometrical absorbance measured at 450nm. The absorbance value obtained for each standard was plotted against concentration using the computer program MultiCalc, creating a standard curve. The concentration of IL-2 protein in the culture supernatant was determined from the standard curve.

2.2.9 Statistical analysis

The Mann-Whitney rank-sum test was used to evaluate statistical differences between patient groups while the Wilcoxon signed-rank test was used to assess statistical differences between stimulated and unstimulated samples. A p-value of less than 0.05 was considered statistically significant.

2.3 Results

2.3.1 TaqMan PCR

Relative quantification was performed by real-time RT-PCR using the TaqMan 7700 system. Results were calculated from the measured Ct values and using the $2^{-\Delta\Delta Ct}$ formula. The biopsy samples stimulated with prolamin were calibrated against their corresponding unstimulated sample to give a ratio or fold-change of expression with regard to the stimulus.

2.3.1.1 IFN-γ

Gliadin

The median fold-change of IFN-γ mRNA expression in coeliac biopsy samples stimulated with gliadin was 2.8 (interquartile range 1.052-4.88), while the median fold-change for control samples stimulated with gliadin was 1.09 (interquartile range 0.681-1.68) (Fig.2.3.1a). Statistically, the probability of the difference between the two groups being due to chance alone is 0.105.

Five of the seven coeliac samples showed an up-regulation of IFN-γ mRNA when stimulated with gliadin, with four samples displaying an up-regulation of >2-fold. Three out of eight control samples showed an up-regulation when stimulated with gliadin with only 1 sample showing an up-regulation of >2-fold. This was the only sample to have a fold-change greater than the coeliac median.

Hordein

Stimulation of coeliac biopsy samples with hordein resulted in a median fold-change of IFN-γ mRNA expression of 3.27 (interquartile range 0.643-6.195) while stimulation of control biopsy samples gave a median fold-change of 0.276 (interquartile range 0.018- 1.622) (Fig.2.3.2a). There is a 91.5% chance (p=0.085) that the difference between the two patient groups was not a random event.

Four of the seven coeliac samples displayed an up-regulation of IFN-γ mRNA when stimulated with hordein, all of which were increases of >2-fold. Three out of seven control samples were found to have up-regulated IFN-γ mRNA though none of the responses were >2-fold and none were greater than the coeliac median.

Secalin

Coeliac biopsy samples stimulated with secalin exhibited a median fold-change of IFN-γ mRNA expression of 3.36 (interquartile range 1.316-64.09) while that for control samples stimulated with secalin was 1.59 (interquartile range 0.209-3.882) (Fig.2.3.3a). The probability of the difference between the two sample groups being due to chance is 0.294.

Six out of eight coeliac samples showed an up-regulation of IFN-γ mRNA when stimulated with secalin, with five samples having an up-regulation of >2-fold and two of these samples having marked up-regulation of 84.01-fold and 193.75-fold. Four of the eight control samples showed an up-regulation of >2-fold when stimulated with secalin, only two of which were greater than the coeliac median.

2.3.1.2 IL-2

Gliadin

Stimulation of coeliac biopsy samples with gliadin resulted in a median fold-change of IL-2 mRNA expression of 0.52-fold (interquartile range 0.182-2.0) while stimulation of control samples manifested a median fold-change of 0.64 (Fig.2.3.4a).

Two of the seven coeliac samples showed an up-regulation of IL-2 mRNA expression when stimulated with gliadin, with both of these samples displaying increases of >2-fold. Two out of eight control samples revealed up-regulation when stimulated with gliadin, and both of these responses were >2-fold.

Hordein

Coeliac biopsy samples stimulated with hordein induced a median fold-change of IL-2 mRNA expression of 0.599 (interquartile range 0.462-43.07), while that for control samples stimulated with hordein was 0.633 (interquartile range 0.024-5.39) (Fig.2.3.5a)

Three out of seven coeliac samples exhibited up-regulation of IL-2 mRNA expression when stimulated with hordein, all three having increases of >2-fold. Three out of seven control samples showed up-regulation when stimulated with hordein, with two samples displaying up-regulation of >2-fold.

Secalin

The median fold-change of IL-2 mRNA expression in coeliac biopsy samples stimulated with secalin was 1.94 (interquartile range 1.58-6.625), while control samples stimulated with hordein had a median fold-change of 1.31 (interquartile range 0.166-2.4) (Fig.2.3.6a).

Seven of the eight coeliac samples demonstrated up-regulation of IL-2 mRNA expression when stimulated with secalin, with four samples showing up-regulation of >2-fold. Four of the eight control samples displayed up-regulation when stimulated with secalin, three of which had up-regulation of >2-fold.

2.3.2 ELISA

The supernatants taken from organ culture of biopsy samples were analysed for the expression of proteins. The results were calculated from a standard curve (Fig. 2.3.7a and b) and protein concentrations were determined for each sample. The response to the stimulated supernatant was divided by the response to the unstimulated supernatant to give a fold-change of expression with regard to the stimulus.

2.3.2.1 IFN-γ

Gliadin

Two of the seven coeliac supernatant samples showed an increased IFN-γ protein response when stimulated with gliadin (Fig.2.3.1b). The remaining coeliac samples and all the control samples had undetectable levels of protein. The two coeliac supernatant samples that displayed increased protein expression correlated to biopsy samples that had an up-regulation of IFN- γ mRNA expression (2.8 and 4.6-fold).

Hordein

One coeliac and one control supernatant sample had detectable levels of IFN-γ protein when stimulated with hordein, though neither showed up-regulation of gene expression (Fig.2.3.2b). The remaining coeliac and control supernatant samples were below the quantification level when stimulated with hordein.

Secalin

IFN-γ protein expression was not detected in coeliac or control supernatant samples when stimulated with secalin (Fig.2.3.3b).

2.3.2.2 IL-2

Gliadin

One coeliac supernatant sample showed up-regulation of protein expression when stimulated with gliadin and this sample correlated to the biopsy sample with the highest increase in IL-2 mRNA (Fig.2.3.4b). The remaining coeliac samples were below the limit of detection. Two of the control supernatant samples had IL-2 protein expression levels that were detectable, however, they did not show up-regulation of gene expression.

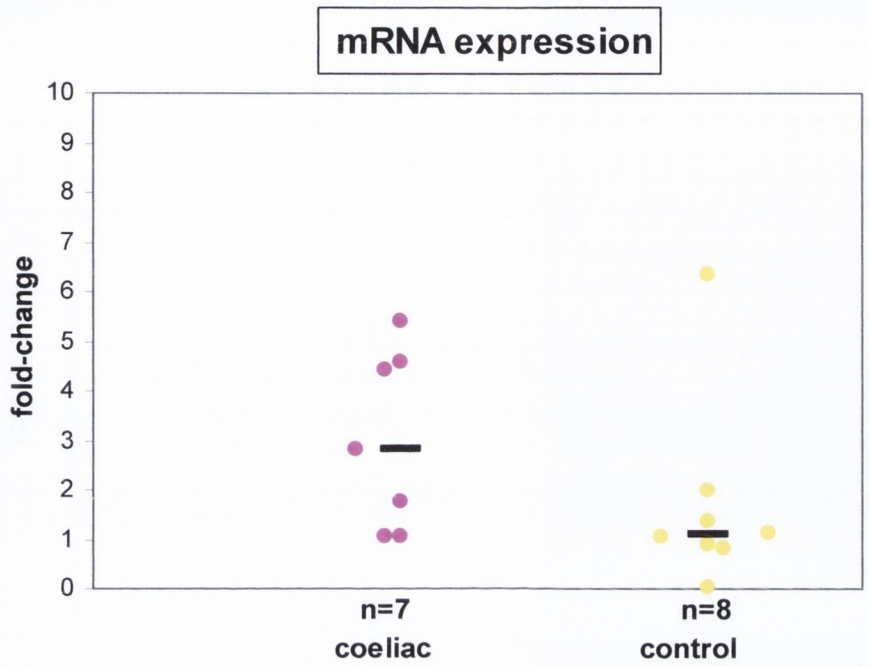
Hordein

When stimulated with hordein, three out of seven coeliac supernatant samples expressed detectable levels of IL-2 protein (Fig.2.3.5b). Of these three samples, two showed up-regulation of >2-fold. IL-2 protein was also detected in all seven control supernatant samples, however, none of these samples showed up-regulation of >2-fold.

Secalin

Seven of the eight coeliac supernatant samples had detectable levels of IL-2 protein expression when stimulated with secalin (Fig.2.3.6b) with only one sample demonstrating up-regulation of >2-fold. Seven out of eight control supernatant samples were found to have detectable levels of IL-2 protein, none of which showed up-regulation of protein expression >2-fold.

A.



B.

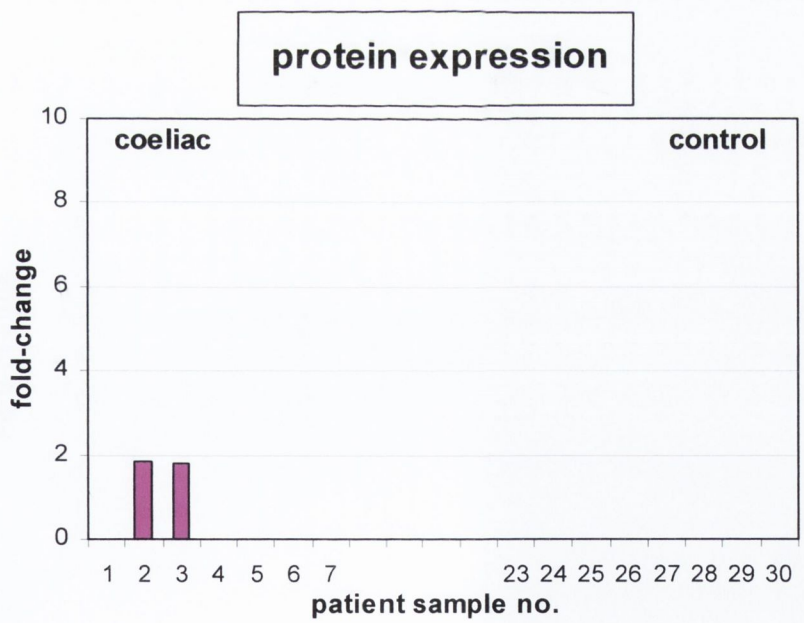
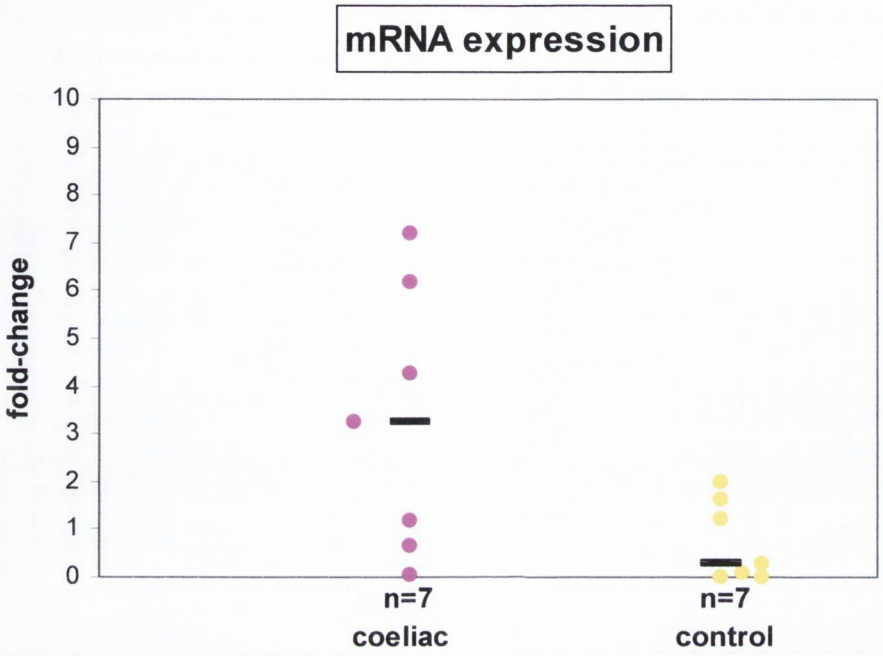


Figure 2.3.1: IFN-γ expression in coeliac and control duodenal tissue following culture with gliadin. Figure A: mRNA expression; median values for each group are shown as horizontal bars. Figure B: protein expression.

A.



B.

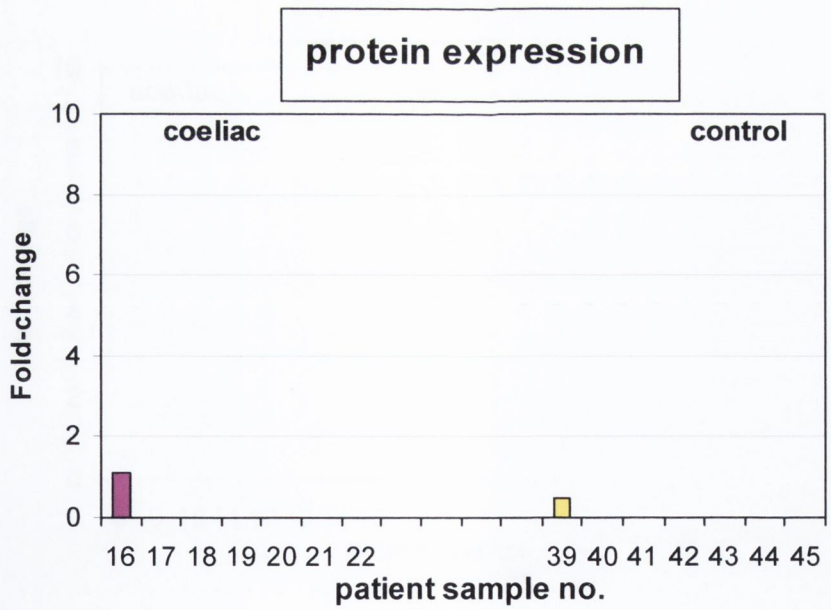
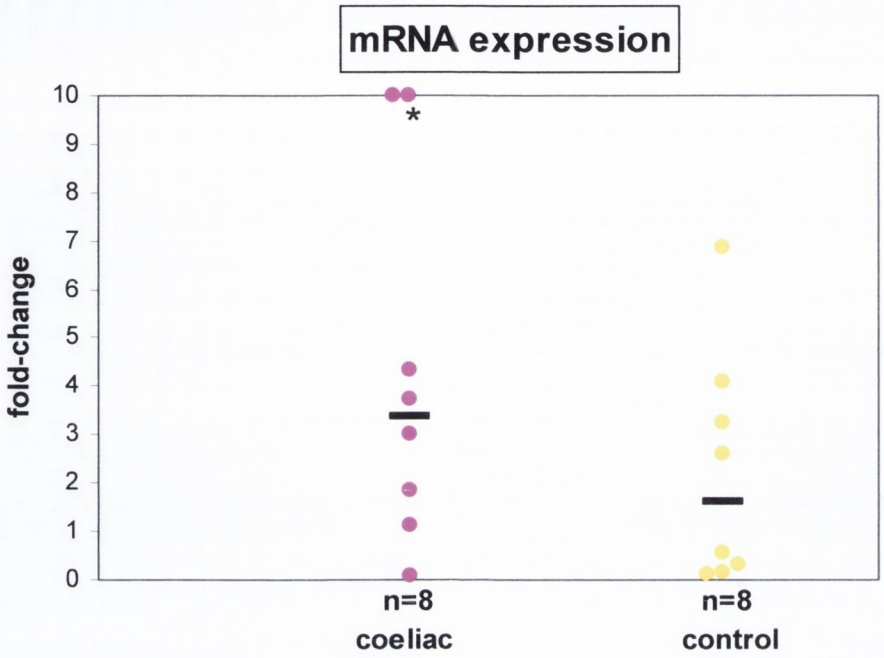


Figure 2.3.2: <u>IFN-γ</u> expression in coeliac and control duodenal tissue following culture with <u>hordein</u>. Figure A: mRNA expression; median values for each group are shown as horizontal bars. Figure B: protein expression.

A.



B.

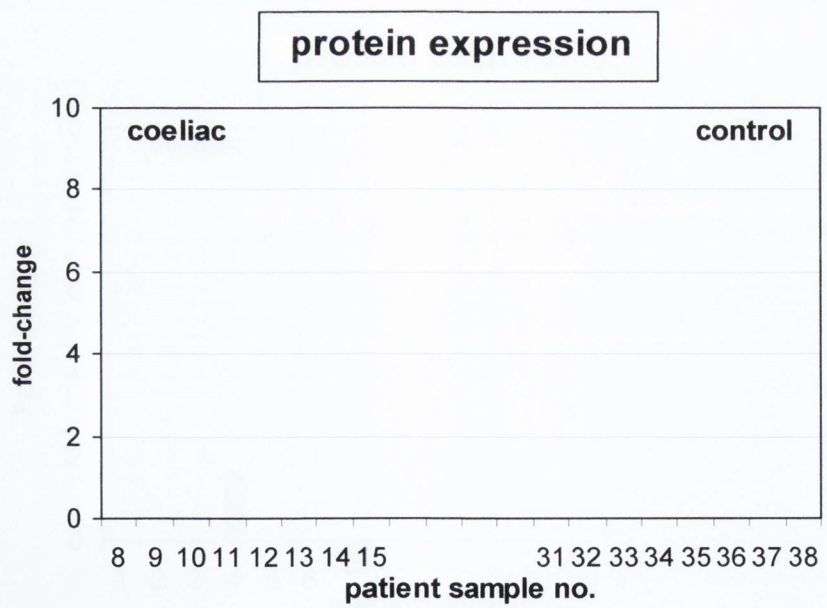
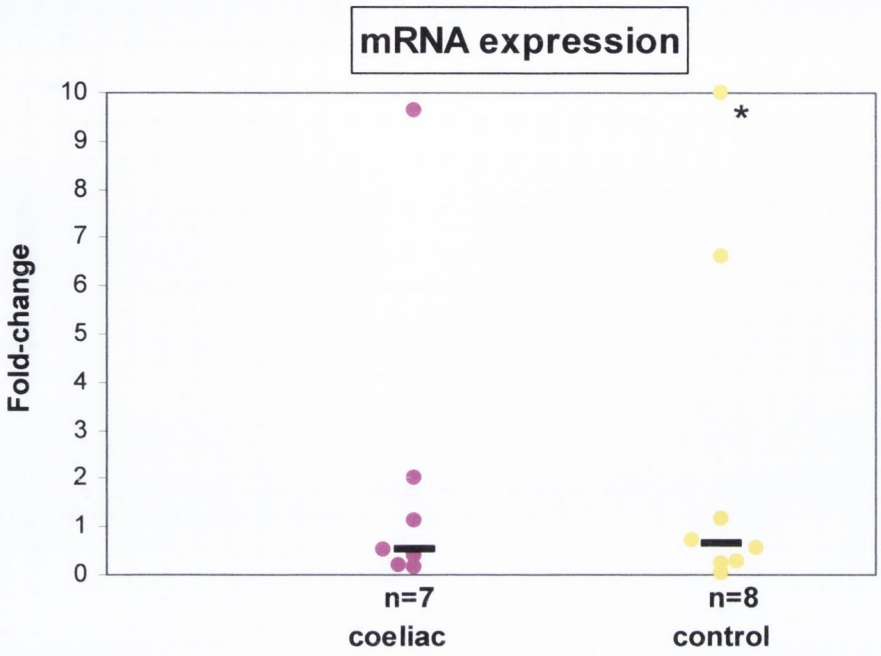


Figure 2.3.3: <u>IFN-γ</u> expression in coeliac and control duodenal tissue following culture with <u>secalin</u>. Figure A: mRNA expression; median values for each group are shown as horizontal bars, * = values > 10-fold. Figure B: protein expression.

A.



B.

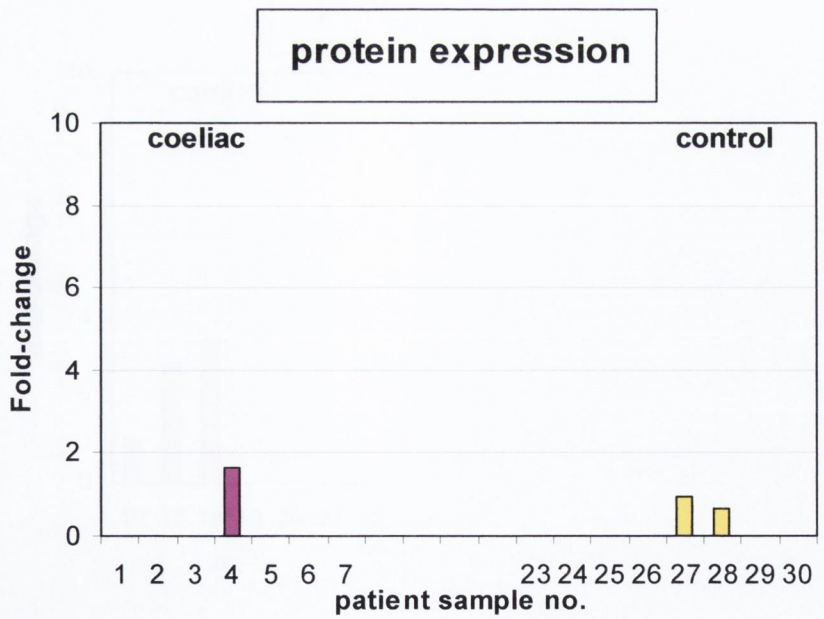
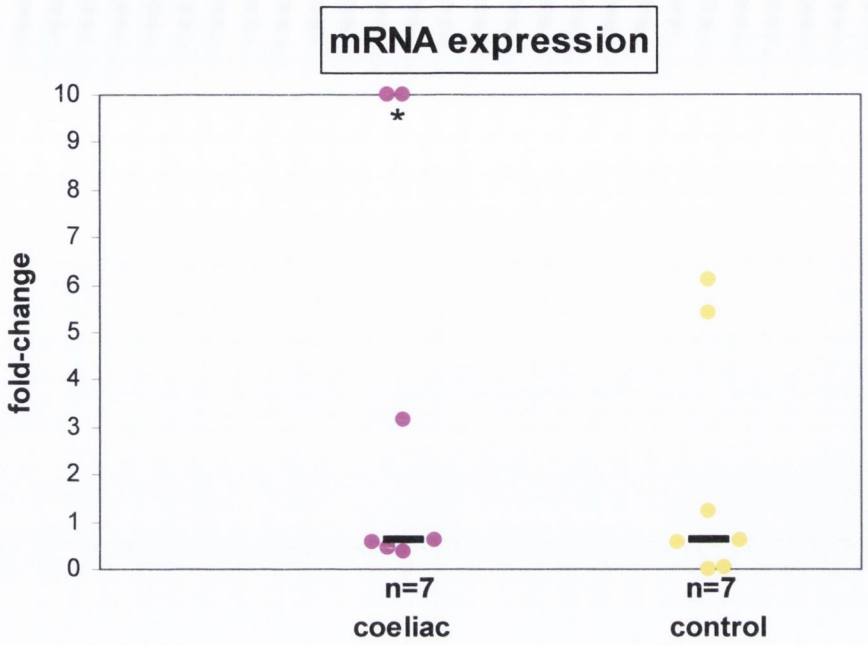


Figure 2.3.4: <u>IL-2</u> expression in coeliac and control duodenal tissue following culture with <u>gliadin</u>. Figure A: mRNA expression; median values for each group are shown as horizontal bars, * = value >10-fold. Figure B: protein expression.

A.



B.

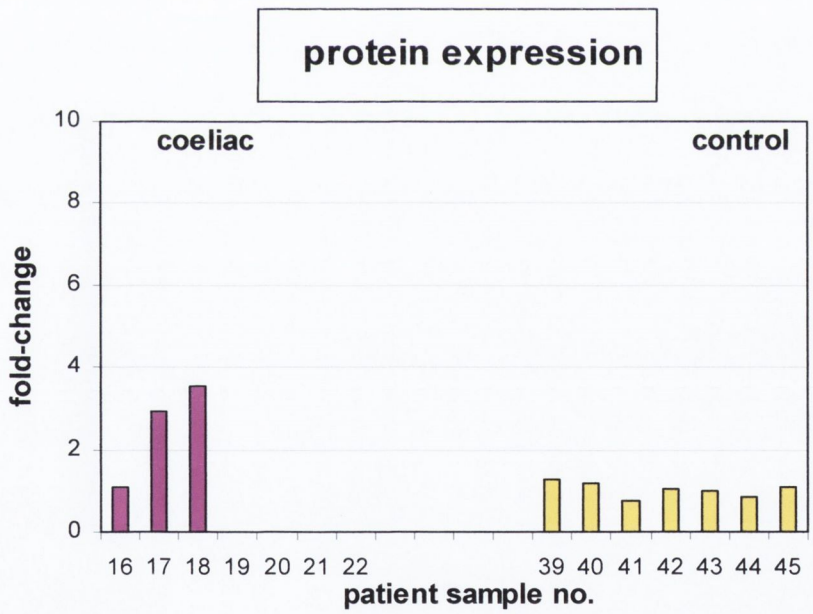
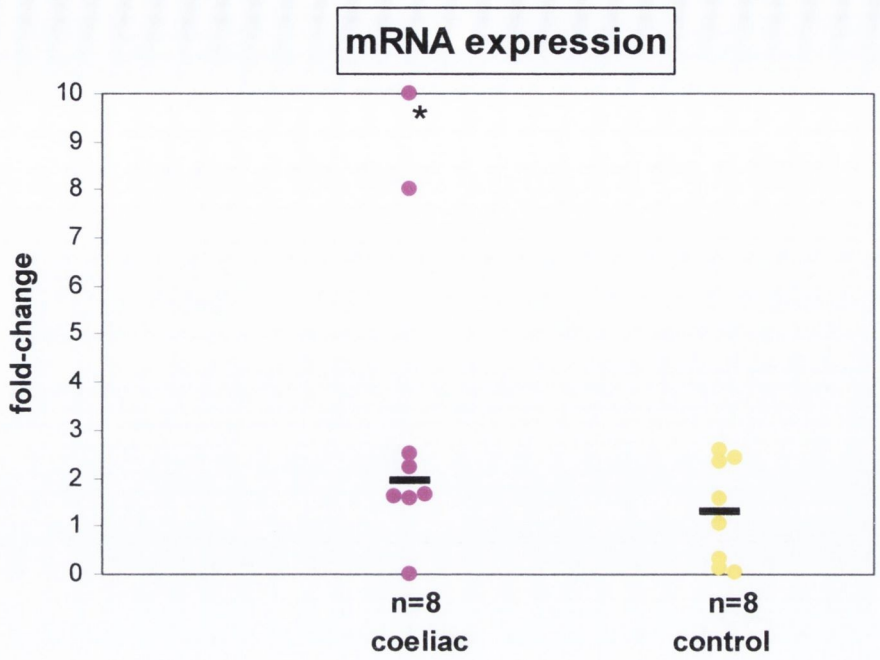


Figure 2.3.5: <u>IL-2</u> expression in coeliac and control duodenal tissue following culture with <u>hordein</u>. Figure A: mRNA expression; median values for each group are shown as horizontal bars, * = value >10-fold. Figure B: protein expression.

A.



B.

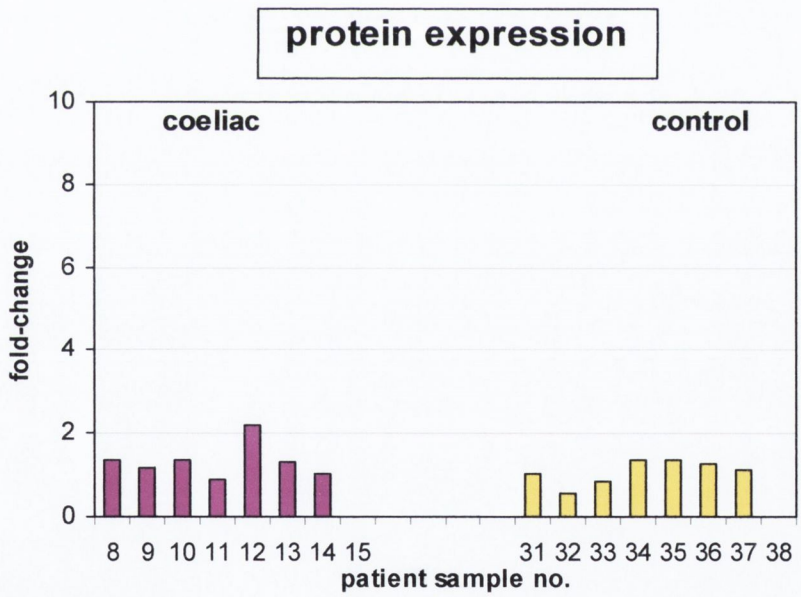


Figure 2.3.6: <u>IL-2</u> expression in coeliac and control duodenal tissue following culture with <u>secalin</u>. Figure A: mRNA expression; median values for each group are shown as horizontal bars, * = value >10-fold. Figure B: protein expression.

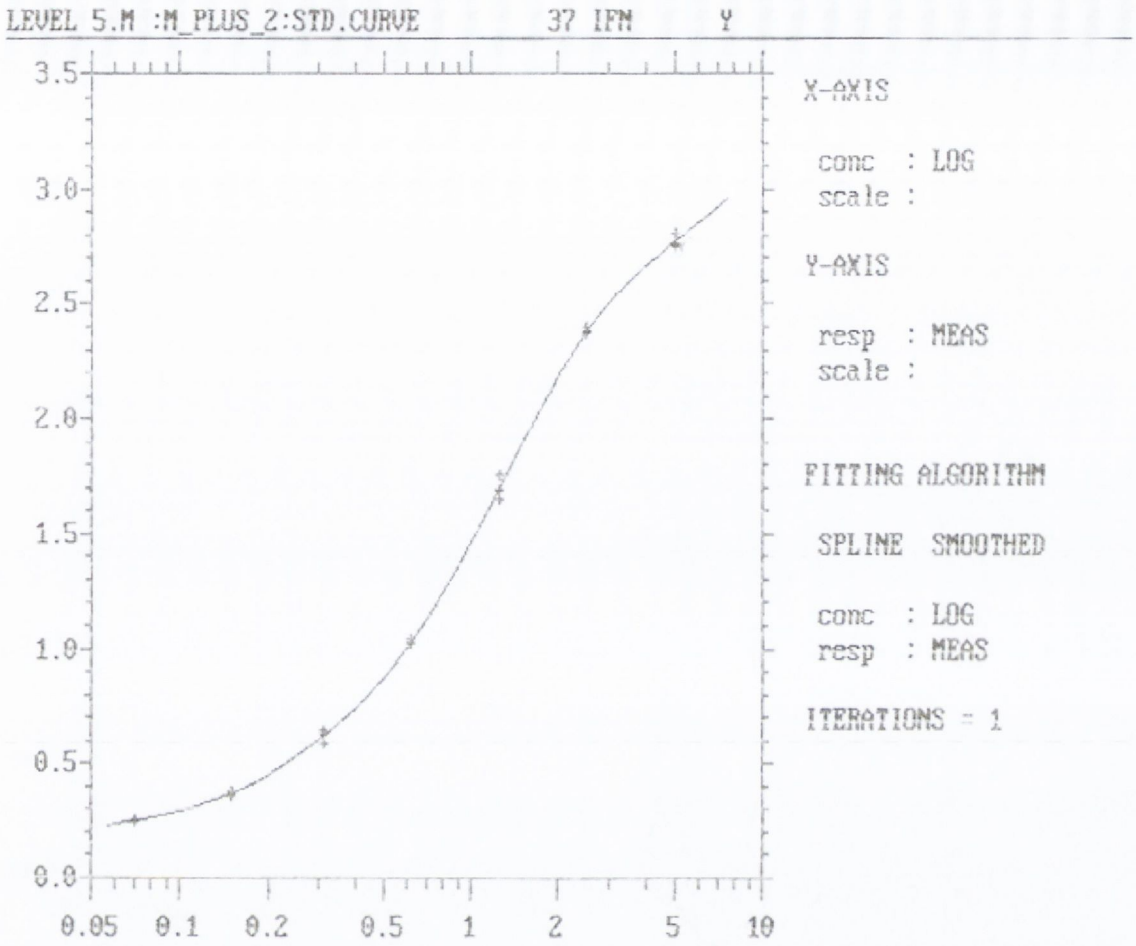


Figure 2.3.7a: IFN-γ ELISA standard curve. X-axis shows the absorbance of standards (A405nm), Y-axis shows the concentration of standards in ng/ml.

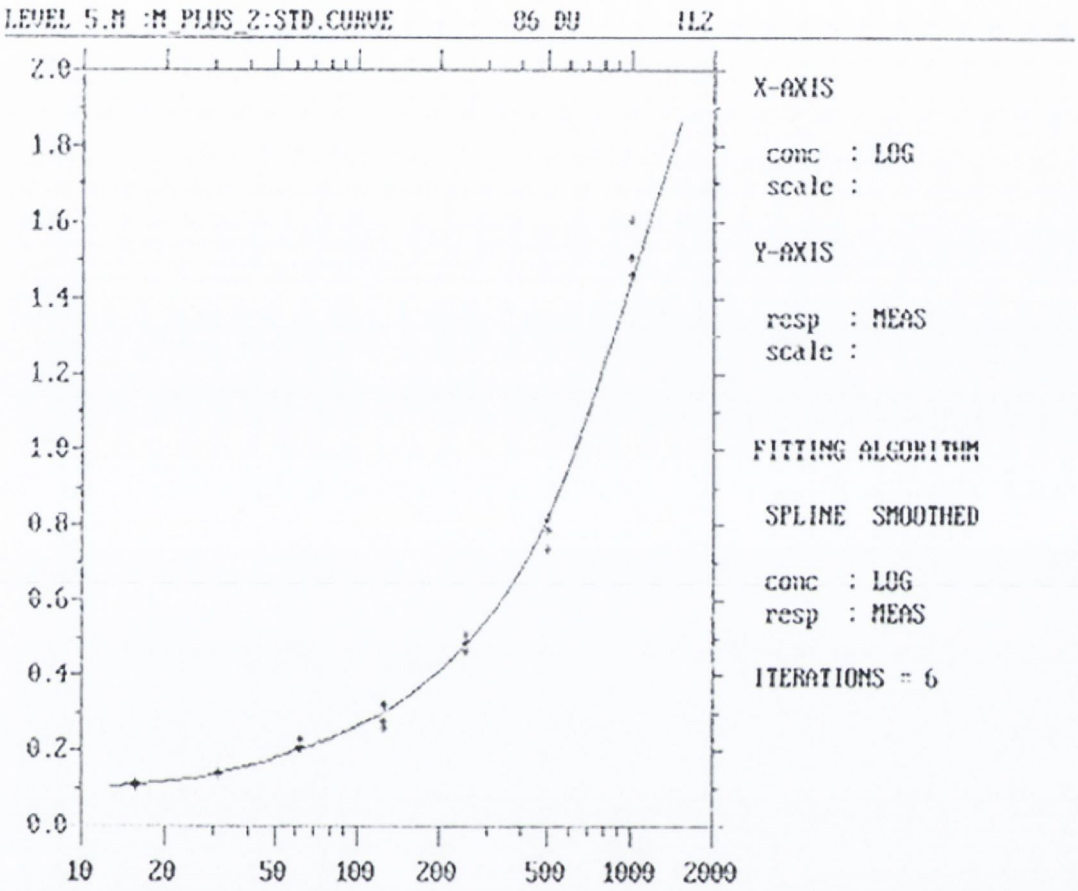


Figure 2.3.7b: IL-2 ELISA standard curve. X-axis shows the absorbance of standards (A450nm), Y-axis shows the concentration of standards in pg/ml.

2.4 Discussion

Barley and rye have long been excluded from the gluten-free diet along with wheat, even though only limited evidence to their toxicity exists. Members of the same tribe as wheat (hordeae; Fig. 1.3.1), these cereals have similar amino acid composition which was assumed to give them similar toxic properties to wheat. Recently, Vader *et al.* [2003b] have shown that immunogenic peptides from barley and rye can stimulate T-cell lines/clones *in vitro.* The purpose of this study was to examine the effect of barley, rye and wheat *ex vivo* on patients with coeliac disease and determine whether immune activation does occur in response to these cereals.

All three cereal prolamins (hordein, secalin and gliadin) induced up-regulation of IFN-γ mRNA in biopsies from coeliac patients when compared to biopsies from control subjects. The cytokine mRNA results were expressed as a fold-change i.e. the ratio between the cultured biopsies' response to a prolamin compared to the response to culture medium alone. Hordein caused the greatest increase in IFN-γ mRNA with a median 3.27-fold increase compared to 0.28-fold change in the control subjects. Secalin also elicited an increased IFN-γ mRNA response, with a median 3.36-fold increase compared to 1.59-fold in the control group. Finally, in agreement with previous studies [Nilsen *et al.* 1998; Kilmartin *et al.* 2003], gliadin also induced an mRNA IFN-γ response in coeliac biopsies (2.82-fold increase) in contrast to the control subjects (1.09-fold increase).

IL-2 mRNA was also measured after culture with the cereal prolamins. Although up-regulation was seen in a few individual patient samples in response to gliadin and hordein, no difference was observed between median fold-changes of coeliac and control samples. Secalin induced some up-regulation of IL-2 mRNA in coeliac biopsies (median fold-change 1.94) but this was mirrored by slight up-regulation in control biopsies (median fold-change 1.31).

The time-point chosen for this study (4 hours) has been previously shown to be optimal for cytokine mRNA production [Nilsen *et al.* 1998]. However, it is known that cytokine protein is optimally produced at a later time-point i.e. more than 20 hours

[Beckett *et al.* 1999; O'Keeffe *et al.* 1999]. Hence, although IFN-γ and IL-2 protein production was investigated in this study, the finding that the protein levels were undetected in many of the samples was not unexpected. In addition, it is known that cytokines exert autocrine and paracrine effects and the proteins may become bound to receptors shortly after being expressed. Previous studies have also found poor cytokine protein expression at this time-point [Nilsen *et al.* 1998; Kilmartin *et al.* 2003]. Nonetheless, after four hours of culture with gliadin, IFN-γ protein was detected in the culture supernatants of two coeliac patients and IL-2 protein in the supernatant of a further coeliac patient. Interestingly, these patients had high levels of the corresponding cytokine mRNA. It is possible that cytokine protein was produced by biopsies from other patients but the levels were too low for detection.

An up-regulation of IFN-γ mRNA in response to gliadin has been reported in many studies. Nilsen *et al.* [1998] found that the expression of IFN-γ mRNA was increased in biopsies from treated coeliac patients cultured with gluten, as well as in untreated coeliac patients ingesting gluten in their diet, when compared to control patients. Kontakou *et al.* [1995] showed an increase in IFN-γ mRNA in treated coeliac patients challenged *in vivo.* Westerholm-Ormio *et al.* [2002] and Forsberg *et al.* [2002] also demonstrated an up-regulation of IFN-γ mRNA in untreated coeliac patients compared to controls. Furthermore, a marked IFN-γ mRNA response has been shown in studies examining T-cells cloned from duodenal biopsies of coeliac patients [Przemioslo *et al.* 1995; Nilsen *et al.* 1995; Troncone *et al.* 1998]. The findings of our study with gliadin are in agreement with these reports and show that IFN-γ mRNA is an appropriate marker of prolamin-induced immune activation in coeliac disease.

The finding that the pattern of IL-2 expression did not follow IFN-γ expression may at first seem strange given that both these pro-inflammatory cytokines are produced by Th1 cells and are often found co-expressed [Spellberg and Edwards 2000, Sánchez *et al.* 2004]. On closer examination of the literature, however, it is clear there are many situations where IFN-γ is up-regulated and IL-2 is not. Nilsen *et al.* [1998] and Forsberg *et al.* [2002] showed that tissue samples from untreated coeliac patients displayed high levels of IFN-γ and low levels of IL-2. Furthermore, Forsberg *et al.*

[2002] demonstrated that the levels of IL-2, after *in vivo* challenge with gluten, were slightly lower than the levels found in coeliac patients who were on a gluten-free diet. Low IL-2 production associated with elevated IFN-γ has been observed in other conditions with chronic immune activation such as inflammatory bowel disease [Kusugami *et al.* 1991], chronic hepatitis C virus (HCV) infection [Semmo *et al.* 2005] and human immunodeficiency virus (HIV) infection [Fan *et al.* 1993]. Long-term exposure of $CD4^+$ T-cells to IFN-α results in a decreased production of IL-2 and decreased proliferation of $CD4^+$ T-cells in response to IL-2. This is thought to occur by down-regulation of surface CD3 and CD28, decreased MEK1/ ERK1/2 phopshorylation and down-regulation of the α-chain of the IL-2R complex [Zella *et al.* 2000]. Over-expression of IFN-α has been shown to occur in the mucosa of untreated coeliac patients which in turn enhances IFN-γ synthesis [Monteleone *et al.* 2001]. Over-production of IFN-α has also been reported in HIV infection and correlates with disease progression [Gringeri *et al.* 1996]. Spontaneous secretion of cytokines is known to occur in the normal healthy gut which maintains a state of controlled activation [Lundqvist *et al.* 1996; Forsberg *et al.* 2002]. Thus, the reduction of IL-2 production in chronic diseases may be explained by over-stimulation of CD4+ T-cells. These T-cells proliferate in response to antigen but lose their IL-2 secretory capacity as they acquire effector characteristics [Semmo *et al.* 2005]. Finally, the production of cytokine mRNA in cells could also be altered by epigenetic regulation of gene transcription such as DNA methylation and the binding of transcription factors to *cis*-regulatory regions in the promotor of the gene [Ansel *et al.* 2003].

Although the patients recruited for this study were all placed on treatment, some patients showed evidence of not following a strict gluten-free diet with five patients testing positive or weak-positive for anti-endomysial antibodies (EMA). The degree of mucosal recovery also varied amongst the patient group, with two patients showing total villous atrophy (grade 3c; section 1.2.2) while many others had no sign of any damage (grade 0). The IFN-γ response, however, did not correlate with severity of mucosal damage or antibody status. Thus, for example, patients with a grade 0 lesion (section 1.2.2) in some cases showed a high IFN-γ response and in other cases showed a low or non-existent IFN-γ response. The two highest overall IFN-γ responses, found after culture with secalin, had grade 0 and grade 1 histology, while of the two patients

with grade 3c histology, one produced an IFN-γ response and the other did not. These results are in contrast with those described by Wapenaar *et al.* [2004] who reported that IFN-γ mRNA production correlated with the degree of tissue damage. This disparity may be explained by the differences in technique used; Wapenaar *et al.* recruited both treated and untreated coeliac patients and used their biopsies directly to measure IFN-γ mRNA levels rather than performing organ culture. Their method of calibration also differed to this study where stimulated samples were calibrated against unstimulated samples from the same patients: Wapenaar *et al.* calibrated their results against the mean generated for the control patients. They did, however, describe a great variability of expression levels within their patient groups which is similar to the results seen in this study. The levels of IL-2 mRNA produced in response to the three prolamins also showed no correlation to EMA status or mucosal damage.

The organ culture system has long been used in investigating the effect of cereals in coeliac disease. Previously, many studies measured morphological parameters such as a decrease in enterocyte height [Howdle *et al.* 1981; Fluge *et al.* 1981] and an increase in intra-epithelial lymphocytes [Bramble *et al.* 1981] or stained immunohistochemically for markers such as CD (cluster of differentiation) molecules and HLA molecules [Maiuri *et al.* 1996; Fais *et al.* 1992]. These results rely on sizable, well-orientated biopsies which, in recent years with the increasing use of endoscopic forceps rather than the Crosby capsule to obtain biopsies, are difficult to obtain. A degree of observer subjectivity is also inevitable. For this reason we chose to examine the immune response within the tissue, investigating a well documented inflammatory cytokine response [Kontakou *et al.* 1995; Nilsen *et al.* 1998; Westerholm-Ormio *et al.* 2002; Forsberg *et al.* 2002; Kilmartin *et al.* 2003], by measuring mRNA and protein production with an objective and quantitative read-out process in the form of real-time PCR and ELISA. A disadvantage of this system is the large number of biopsies needed to produce sufficient cytokine mRNA for detection. Thus, while it would be preferable to examine responses to all prolamins in each patient, it would not be ethical to take the number of biopsies required for this experiment.

Although the statistical values were outside the common cut-off ($p \leq 0.05$), the results from this study show a clear difference between the coeliac and control IFN-γ mRNA responses, which must be considered clinically relevant. Unexpectedly, some of the controls produced a cytokine mRNA response to the prolamins. It should be noted that the control patients were disease control patients, who were attending the gastroenterology clinic for a variety of complaints such as oesophagitis or colonic polyps as well as patients in whom no abnormal gastrointestinal features could be found. While all of these patients had normal duodenal mucosa when tested, it is possible that their gastrointestinal system was altered and perhaps made more susceptible to challenge with antigen. Although it would be preferable to recruit healthy controls from the general population, it would not be ethically justifiable to perform such an invasive procedure without medical reason.

Amongst the coeliac patient samples, there was variability in expression of cytokines. The amount of gluten that can be ingested by coeliac patients before an adverse reaction occurs appears to vary from patient to patient [Collin *et al.* 2004]. Similarly, it is possible that the concentration of gluten in organ culture and the length of culture time required to invoke a cytokine response from a sample may vary from patient to patient. Both Forsberg *et al.* [2002] and Wapenaar *et al.* [2004] demonstrated considerable variation between patients in the levels of cytokines expressed.

From previous in situ hybridisation studies, it is known that the $CD4^+$ lamina propria T-cells are the main source of IFN-γ and IL-2 [Kontakou *et al.* 1995; Nilsen *et al.* 1998] and it is likely that these cells are the source of the cytokine production in the biopsy samples used in this study. It cannot be discounted, however, that other cells in the heterogeneous biopsy sample may produce these cytokines and/or may produce other signals that interfere with Th1 cytokine production. One study that compared cytokine mRNA production by mucosal biopsy specimens to isolated autologous lamina propria mononuclear cells found variation in the levels of cytokines measured between the two tissue sources [Fukushima *et al.* 1995]. They also reported differing levels of cytokine mRNA in closely adjacent biopsies, perhaps due to differing numbers of mononuclear cells in different biopsies. It should also be noted that the

technique of culturing biopsies which exposes the normally protected lamina propria of the tissue directly to antigen may facilitate lymphocyte activation more readily.

RT-PCR relative quantification relies on a normalisation of the gene transcript of interest to a housekeeping gene. This is performed in order to take into account differing amounts of mRNA in each sample. Thus, when a large amount of the gene of interest is found, it is safe to assume this is not simply due to the sample containing more mRNA. A housekeeping gene is one which should be constitutively expressed and not regulated in the cells being studied. In this study we used the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an endogenous control, which was the recommended housekeeping gene at the time of initiation of this study. Since then, the gene has come under scrutiny with reports of variation in expression levels between different tissue types and between different patient samples [Barber *et al.* 2005]. In this study, however, the comparison is made between tissue samples from the same patient (unstimulated and stimulated) and the samples came from the same tissue source, obtained at the same time. Moreover, a number of recently published studies on coeliac intestinal tissue have used GAPDH to normalise when performing quantitative RT-PCR [Forsberg *et al.* 2002; Meresse *et al.* 2004; Martín-Pagola *et al.* 2004]. Furthermore, Forsberg *et al.* report that they found no difference in GAPDH mRNA levels between patient groups or between different cell populations within the same patients.

In conclusion, this study demonstrated that barley, rye and wheat prolamins induce an immune response in coeliac patients *ex vivo* with an increased production of IFN-γ mRNA but not of IL-2 mRNA. However, as statistical significance could not be reached with these experiments, this system may not be optimal for the study of immune responses to prolamins. A more optimal system to study immune activation in coeliac disease may be to examine an isolated homogeneous population of cells, and analyse genes which are expressed in a consistent trend in coeliac patients that could act as markers for active disease.

Chapter 3

Examination of the molecular responses of the epithelial cell to the gluten-induced inflammatory environment of the coeliac mucosa, using microarray analysis

3.1 Introduction

Coeliac disease has a strong HLA association with approximately 95% of coeliac patients possessing the HLA-DQ2 molecule (sections 1.1.2.1 and 1.3.1.1). A large population based study showed the disease concordance rate between monozygotic twins to be 75% [Greco *et al.* 2002]. For a multifactorial disease, this rate is considered high, especially in view of the reported concordance rates (in monozygotic twins) of other diseases- 13% in insulin dependent diabetes [Kaprio *et al.* 1992], 12.3% in rheumatoid arthritis [Aho *et al.* 1994], 26.7% for multiple sclerosis [Ebers and Sandovnick 1994] and 20% for Crohn's disease [Thompson *et al.* 1996]. However, the concordance rate for coeliac disease in HLA-matched dizygotic twins is only 17% [Greco *et al.* 2002]. Thus, while the evidence points to a very strong genetic component in coeliac disease, the HLA region only partially contributes to this and other non-HLA-linked genes must have a role.

Presently genome searches for susceptibility loci other than the HLA-region have failed to reach a genome-wide level of significance [Sollid 2002]. This is possibly because each pre-disposing gene has a small effect by itself. Also gene-gene interactions and the heterogeneous distribution of the gene in an out-bred human population could cause the significance to be lessened. The chromosomal regions receiving the most attention are: 2q33 [Holopainen *et al.* 1999; Naluai *et al.* 2000] a region which encodes CD28, cytotoxic T-lymphocyte antigen 4 (CTLA4) and inducible T-cell co-stimulator (ICOS); 5q31-33 [Greco *et al.* 1998; Liu *et al.* 2001] which includes many candidate susceptibility genes such as CD14, Tmp1, Tim, IL12B and the Th2 cytokine cluster; and 19p13.1 [van Belzen *et al.* 2003], a locus with significant genome linkage recently identified in a Dutch cohort of coeliac patients, which has also been strongly linked to Crohn's disease- another inflammatory disease affecting the small intestine [van Heel *et al.* 2005].

Pathology studies in coeliac disease have focused on the role of T-cells and the pro-inflammatory cytokines they produce [Nilsen *et al.* 1995; Nilsen *et al* 1998; Forsberg *et al.* 2002]. It has been assumed that the damage caused to the mucosa is due to the direct toxic effect of Th1-produed cytokines such as IFN-γ [Wapenar *et al.* 2004] or by

the influence of these cytokines on other cells to produce damaging components such as matrix-metalloproteinases (MMPs) [Daum *et al.* 1999]. Whether the epithelial cell has a role in the pathogenesis is unclear. The sequence of events in the adaptive immune response to gliadin shows the epithelial cell to be involved in the toxicity following T-cell activation. However, recent studies have shown that gliadin can directly affect epithelial cells and that these cells may have a direct role in an innate immune response through their regulation of HLA-DR, MICA and IL-15 expression (Section 1.3.2.3). The position of the epithelial cell as a bystander or a contributor remains to be determined.

Until recently, research into genes with a potential influence in coeliac disease pathogenesis relied on hypothesis-driven candidate gene analysis; however, this has limited the scope of discovery to known genes and pathways. Microarray-based techniques allow for the simultaneous analysis of expression of thousands of gene transcripts, in a hypothesis-free manner [Schreiber *et al.* 2000]. The scientific principle underlying microarray technology is complementary hybridisation between nucleic acids. mRNA from a given tissue is used to generate fluorescently-labelled cDNA, termed the probe, which is hybridised in parallel to a large number of DNA sequences, immobilised on a solid surface in an ordered array. Analysing the microarray with high-resolution fluorescent scanners allows assessment of the fluorescent signal strength that originates from the target-bound probe. This signal is presumed to be directly proportional to the abundance of the RNA species present in the investigated samples.

The aim of this study was to develop a high-throughput gene expression analysis system in order to examine the role of the epithelial cell in the gluten-induced inflammatory environment of the coeliac lesion. Epithelial cells were isolated from biopsies taken from coeliac patients with active disease and compared to controls. Gene transcripts that were significantly up- or down-regulated in the coeliac samples were further analysed for functional relevance.

3.2 Materials and Methods

3.2.1 Patients

Eight duodenal biopsy specimens were obtained from each patient via oesophago-gastro- duodenoscopy. Five patients had active, untreated coeliac disease and five patients undergoing endoscopy for investigation of upper gastrointestinal symptoms were used as a control group. The control patients were sex-matched, age-matched, and where possible pathology-matched to the coeliac patients. Clinical details are outlined in table 3.2.1. Ethics approval for this study was granted by the Joint Ethics Committee Board of St James' Hospital and Tallaght Hospital, Dublin, Ireland.

Table 3.2.1 Patient Clinical Details

Patients	Sex	Age	Duodenal histology	Other histology	Antibody status	Microarray number
Coeliac						
1	Female	61	Type 3a	N/A	tTG +	1
2	Female	45	Type 3b	Barrett's oesophagus	tTG +	2
3	Female	22	Type1	N/A	tTG +	3
4	Female	31	Type 3c	N/A	tTG +	4
5	Female	36	Type 3c	Oesophagus: mild changes of reflux	tTG +	5
Control						
1	Female	58	NDM	Oesophagus: mild changes of reflux	nk	1
2	Female	51	NDM	Oesophagus: moderate changes of reflux	tTG -	2
3	Female	25	NDM	N/A	nk	3
4	Female	30	NDM	Gastric mucosa: superficial acute + chronic inflammation	nk	4
5	Female	26	NDM	Oesophagus: mild chronic inflamation	tTG -	5

tTG, tissue transglutaminase antibody; nk, not known.
Histology grade: 0=normal, 1=raised intraepithelial lymphocytes (IELs), 2= increase in IELs with crypt hyperplasia, 3a= increase in IELs, crypt hyperplasia, mild villous atrophy, 3b= increase in IELs, crypt hyperplasia, marked villous atrophy and 3c= total villous atrophy, NDM=normal duodenal mucosa.

3.2.2 Isolation of epithelial cells

Biopsies were placed in calcium and magnesium free HBSS (Gibco BRL, Scotland) containing 1mM dithiothreitol (DTT) (Sigma, USA) and 1mM EDTA (Sigma, USA) and incubated in a rotating hybridization oven at 37°C for 40 minutes. The supernatant containing the epithelial cells was removed. This was twice washed with calcium and magnesium-free HBSS and centrifuged at 800rpm for 10 minutes before being resuspended in 1 ml of medium.

Cells were diluted 1:5 in Trypan Blue dye and then counted in a haemocytometer. Viable cells stained white while non-viable cells appeared blue. Cells were passed through a 50μm filter to remove aggregates of cells.

3.2.3 Depletion of T-cells

After isolation, the epithelial cells were centrifuged at 12,000rpm and resuspended in 80μl of separation buffer (1M PBS; pH7.2, 5% fetal calf serum and 2mM EDTA). For every 10^6 of cells, 2μl of MACS CD3 Microbeads (Miltenyi Biotech, Germany) was added and mixed. The sample was incubated for 20 minutes at 6°C and then washed using 1ml of separation buffer and centrifugation at 12,000rpm. The sample was then resuspended in 500μl of separation buffer. The LD Depletion Column (Miltenyi Biotech, Germany) was placed in the MidiMACS magnet (Miltenyi Biotech, Germany) and primed with 2ml of cold separation buffer before use. The magnetically labelled cell suspension was applied to the column, allowed to pass through and collected in a sterile tube embedded in ice. The column was then washed 3 times with 3mls, 2mls and 1ml of separation buffer. The eluate was collected, washed and resuspended in 1ml of RPMI. Cells were counted as described in section 3.2.2. The cell suspension was adjusted to a concentration of 1 x 10^6cells/ml.

3.2.4 Flow cytometric analysis

The epithelial cells were separately stained with the following mouse monoclonal antibodies: Fluorescein (FITC)-labelled Ber-EP4 (DAKO, Denmark), FITC-labelled CD3 and FITC/Phycoerythrin (PE) dual-labelled control IgG1/IgG2a (BD, Belgium). The cells (1 x 10^5) were incubated with 5 μl of the antibodies for 30min at 4°C and

then washed twice with PBS (1% BSA). Cells were fixed in 0.5 ml of 0.5% paraformaldehyde (BDH, UK).

Flow cytometric analysis was performed using a FACScan analyser (Becton Dickinson, Belgium) and CellQuest software (Becton Dickinson, Belgium) with the settings listed in Appendix I. Ten thousand cells were acquired for each sample. These were viewed on the basis of size (forward scatter; FSC) and granularity (side scatter, SSC) and gates drawn around the relevant population. The positively stained cells were visualised on an FL1/FL2 dot plot. Quadrants were set using the isotype matched control antibody IgG1/IgG2a and percentages of positively stained cells were then calculated.

3.2.5 RNA extraction

Total RNA was extracted from the epithelial cells using the NucleoSpin® RNA II kit (BD Biosciences Clontech, UK). Cells were lysed in 350µl of denaturing buffer RA1 in combination with 3.5µl of β-mercaptoethanol, vortexed vigourously and frozen at -80°C. Once thawed, the sample was filtered using the provided filter unit and 350µl of 70% ethanol was added. This mixture was loaded onto a NucleoSpin column and centrifuged at 8,000g for 30 seconds. The flow-through was discarded. Membrane desalting buffer was added to the column, centrifuged at 11,000g for 1 minute and the flow-through discarded. Ninety five microlitres of DNase 1 reaction mixture (90% DNase 1 reaction buffer, 10% DNase 1 enzyme) was then loaded onto the column and incubated at room temperature for 15 minutes. Wash buffer RA2 (200µl) was added to the column, centrifuged at 8,000g for 30 seconds and the flow-through discarded. Wash buffer RA3 (600µl) was added to the column, centrifuged at 8,000g for 30 seconds and the flow-through discarded. This step was repeated with 250µl of buffer and centrifuged at 11,000g for 2 minutes. Forty microlitres of RNase-free water was added to the column to elute the RNA and centrifuged at 11,000g for 1 minute. The eluate was reloaded onto the column and centrifuged again. This step was repeated a third time to collect the maximum yield of RNA. The RNA was concentrated further using Microcon YM-30 concentrators (Millipore, Ireland). The sample was loaded onto the filter and centrifuged at 14,000g for 3.5 minutes at 4°C. The filter was turned upside down in a fresh eppendorf and centrifuged at 1,000g for 3.5 minutes at 4°C.

Total RNA was quantified using an Eppendorf Biophotometer. RNA was diluted 1:100 in TE buffer and the spectrophotomter was blanked against TE buffer. Absorbance was read at 260nm to calculate quantity and the 260/280nm ratio was measured to calculate purity of RNA from contaminating protein.

A 1% agarose gel (0.5μg of agarose, 50μl of TBE buffer (0.89M Tris, 0.02M EDTA-Na_2-salt, 0.89M Boric acid) and 5μl of ethidium bromide dye) was used to assess the quality of RNA. Five microlitres of RNA (concentration 0.1μg/μl) was loaded with 1μl loading buffer (0.1% bromophenol blue in 50% glycerol solution) onto the gel and electrophoresed at 120V for 40 minutes. The gel was illuminated using UV light and photographed using Polaroid film. Total RNA was stored at -80°C until use.

3.2.6 Reverse Transcription

Reverse transcription was carried out using the BD Atlas™ SMART™ Fluorescent Probe Amplification Kit (BD Biosciences Clontech, UK). The reaction was optimised using 100ng of total RNA. The total RNA was diluted to 50μl in sterile, deionised water, added to 7μl of 3'SMART CDS Primer II A (12μM) and 7μl of SMART II A Oligonucleotide (12μM) and incubated at 72°C for 5 minutes. A master mix consisting of 20μl of 5X First-strand buffer, 2μl of DTT (100mM), 10μl of cDNA Synthesis dNTP (10mM of each dNTP), 2.5μl of RNasIn (40U/μl, Promega) and 5μl of PowerScript Reverse Transcriptase was added to each RNA sample and the reaction mixture was incubated at 42°C for 1 hour in a hot-lid thermal cycler (PTC-100, Bio-Rad USA). The reaction was stopped by adding 2μl of EDTA (0.5M), and the mixture was stored at -20°C until further use.

3.2.7 Purification of cDNA

cDNA was purified from unincorporated nucleotides before amplification using the CyScribe GFX Purification Kit (Amersham Biosciences, UK). For each cDNA sample, one GFX column was placed in a collection tube and 500μl of capture buffer loaded onto the column. The cDNA was transferred into the GFX column and mixed carefully by gently pipetting up and down. The columns were immediately centrifuged at 13,800g for 30 seconds, and the flow-through discarded from the collection tube. Six

hundred microlitres of 80% ethanol was added to each column and centrifuged at 13,800g for 30 seconds. This wash step was repeated twice and a further centrifugation step was performed for 10 seconds to remove any remaining wash buffer. The columns were transferred into fresh 1.5ml microcentrifuge tubes and 55µl of elution buffer was added directly on top of the glass fibre matrix. Care was taken to ensure the elution buffer completely covered the membrane. The columns were incubated at room temperature for 5 minutes before centrifugation at 13,800g for 1 minute. A further 30µl of elution buffer was added on top of the column, incubated and centrifuged as before. The purified cDNA was collected in the microcentrifuge tube and the column discarded.

3.2.8 Amplification of cDNA

Amplification of cDNA was carried out using the BD Atlas™ SMART™ Fluorescent Probe Amplification kit (BD Biosciences Clontech, UK). The starting total RNA quantity was 100ng; therefore as specified in manufacturer's guidelines, 25µl of purified cDNA was used in the reaction. For each patient sample, two probe samples are needed; thus, two aliquots of 25µl of cDNA were prepared. Each 25µl of sample was added to 55µl of deionised water followed by the addition of a master mix consisting of 4µl of deionised water, 10µl of 10X BD Advantage 2 PCR buffer, 2µl of 50X dNTP (10mM), 2µl of 5'Primer IIA (12µM) and 2µl of 50X BD Advantage 2 Polymerase Mix (BD TITANIUM™ *Taq* DNA polymerase, BD TaqStart™ Antibody, minor amount of proofreading polymerase). The polymerase chain reaction (PCR) was carried out in a thermal cycler (PTC-100, Bio-Rad USA) using the settings described in appendix II-B. For each sample, the optimum number of PCR cycles had to be determined in order to ensure the amplification process was stopped while still in the exponential phase. This was essential as over-cycled cDNA which has reached the plateau phase of amplification could result in a less representative probe when examining differential gene expression. Thus, after 15 cycles of PCR, the reaction was paused and 25µl of sample from each tube was removed and used for optimisation while the remaining 75µl was stored at 4°C. From the 25µl optimisation sample, a 5µl aliquot was further removed and kept for agarose gel analysis. The optimisation sample was returned to the thermal cycler and subjected to an additional 3 cycles. A

5µl aliquot was removed at this point and kept for agarose gel analysis. The optimisation sample was again returned to the thermal cycler and run for an additional 3 cycles and another 5µl aliquot taken for gel analysis. In this way, the reaction was continued up to 27 cycles, after which time, aliquots representing cycles number 15, 18, 21, 24 and 27 were obtained. These aliquots were electrophoresed alongside a 1Kb DNA ladder on a 1.2% agarose/EtBr gel in 1X TBE buffer. The gel was visualised under UV light and photographed using Polaroid film. The optimum number of cycles required for each sample was determined as one cycle fewer than is needed to reach the plateau, as evident on the photographed gel. The samples were retrieved from 4°C storage, returned to the thermal cycler and subjected to the necessary number of additional cycles. Once complete, a 5µl aliquot of each sample was analysed on a 1.2% agarose/EtBr gel under UV light, to ensure the reactions were successful.

The PCR product was purified using the CyScribe GFX Purification Kit (Amersham Biosciences, UK) as described in section 3.2.7, except the purified product was eluted by addition of 50µl of elution buffer. This was collected, re-loaded onto the column and centrifuged twice more in order to ensure a maximal yield. The yield was quantified using an Eppendorf Biophotometer. PCR product was measured undiluted and the spectrophotometer was blanked against elution buffer. Absorbance was read at 260nm to calculate quantity and the 260:280nm ratio was measured to calculate purity.

3.2.9 Synthesis of labelled probes

Labelled probes were synthesised using the BD Atlas™ SMART™ Fluorescent Probe Amplification kit (BD Biosciences Clontech, UK). Two micrograms of purified PCR product was diluted to a final volume of 76µl using PCR-grade water. A master mix of 10µl of 10X Randon Primer Mix (N-18), 2µl of 10X Labelling dNTP (dATP, dCTP, dGTP, dTTP, aminoallyl-dUTP) and 10µl of 10X BD Advantage 2 PCR buffer was added. The reaction was carried out in a thermal cycler using the settings described in appendix II-C and when completed, 0.5µl of EDTA (0.5M; pH 8) and 10µl of QuickClean resin were added to the sample. The mixture was vortexed for 1 minute and spun briefly in a microcentrifuge to collect contents. For each sample, a 0.22µm spin filter was inserted into a collection tube and the sample loaded carefully onto the

filter. This was centrifuged at maximum speed for 1 minute and the aminoallyl-labelled cDNA was collected after the spin filter was discarded. Eleven microlitres of sodium acetate (3M) was added, vortexed and then 275µl of ice-cold ethanol (100%) was added. The sample was vortexed and precipitated overnight at -20°C.

Upon retrieval from -20°C, the sample was centrifuged at maximum speed for 20 minutes at room temperature. The supernatant was carefully removed, the pellet washed with 300µl of ethanol (75%), spun at maximum speed for 10 minutes and air-dried. The pellet was then dissolved in 10µl of 2X Fluorescent Labelling Buffer. Individually sealed, single-use aliquots of monoreactive Cy3-NHS ester and Cy5-NHS ester dyes (Amersham Biosciences, UK) were separately dissolved in 10µl of DMSO in amber-coloured eppendorf tubes. The 10µl cDNA sample was then added to the relevant dye sample. From each patient sample, two samples of probe were prepared; thus, one sample was dissolved in Cy3 dye and the other in Cy5 dye. The sample/dye mixture was incubated for 1 hour at room temperature. After incubation, 2µl of sodium acetate (3M) and 50µl of ethanol (100%) were added, mixed well and the labelled probe was allowed to precipitate for 2 hours at -20°C. The sample was centrifuged at maximum speed for 20 minutes. The supernatant was carefully removed and the pellet washed in 300µl of ethanol (70%), spun and supernatant removed again. The pellet was then dissolved in 100µl of PCR-grade water.

The labelled probe was purified from unreacted dye using the CyScribe GFX Purification kit (Amersham Biosciences, UK) as described in section 3.2.7 with the following exceptions: the kit wash buffer was used rather than 80% ethanol and the labelled probe was eluted in 4 x 50µl of elution buffer. The eluates were combined together and re-eluted through the column one more time. The eluted labelled probe was purified from particulate matter by filtering through a 0.22µm spin filter.

3.2.10 Analysis of labelled probe

The quantity and quality of the labelled probe was determined by UV/visible spectrophotometry using a Genesys 5 spectrophotometer (Thermo Electron Corporation, US). The labelled probe was analysed undiluted and the spectrophotometer was blanked with elution buffer. Absorbance was read at different

wavelengths to assess various parameters (Table 3.2.2). The optimal volume of labelled probe to use in the hybridisation reaction was determined on the basis of absolute optical units (OU_λ), using the following formula: V_{opt} (µl) = $\frac{1{,}000 \times OU_\lambda}{A_\lambda}$

where OU_λ for Cy3 and Cy5 is 0.01 (determined by BD Biosciences Clontech) and A_λ is the measured absorbance maxima for each dye; 550nm for Cy3 and 650nm for Cy5.

Table 3.2.2 Probe analyses

Probe component	**Wavelength (nm)**	**Measurement**
Nucleic acid	260	Estimates amount of cDNA.
Cy3/nucleic acid baseline	450	Broad spectrum "baseline adjustment" for both nucleic acid and Cy3 absorbance. Should be ≤ 15% of A_{550}
Cy5/nucleic acid baseline	520	Broad spectrum "baseline adjustment" for both nucleic acid and Cy5 absorbance. Should be ≤ 15% of A_{650}
Cy3	550	Absorbance maximum for Cy3
Cy5	650	Absorbance maximum for Cy5
Cy3 baseline	650	Baseline adjustment for analysing Cy3 absorbance at 550nm. Should be ≅ 0
Cy5 baseline	750	Baseline adjustment for analysing Cy5 absorbance at 650nm. Should be ≅ 0

3.2.11 Microarray Hybridisation

The microarrays used in this study were Atlas Glass Human 3.8 I microarrays (BD Biosciences Clontech, UK). Five biological replicate experiments were conducted, each one comparing one untreated coeliac sample to one control sample. In each experiment, two technical replicates were performed. Onto one microarray, Cy3-labelled coeliac cDNA and Cy5-labelled control cDNA were co-hybridised. Onto the other microarray, Cy5-labelled coeliac cDNA and Cy3-labelled control cDNA were co-hybridised. In this manner any discrepancies in rates of incorporation of the different dyes during the labelling step are controlled for.

The appropriate coeliac and control probe were combined together and 1μl of Fluorescent SMART blocking solution (from BD Atlas™ SMART™ Fluorescent Probe Amplification kit; BD Biosciences Clontech, UK) added, incubated at 95°C for 5 minutes, and then transferred onto ice for 2 minutes. The denatured probe mixture was added to 2.1 ml of hybridisation solution (BD Biosciences Clontech, UK), prewarmed to 50°C. The probe/hybridisation mixture was carefully pipetted into the hybridisation chamber containing the microarray slide, taking care not to create any air-bubbles. The hybridisation chamber was placed in a test-tube rack and incubated overnight (minimum 16 hours) at 50°C in an upright position.

After incubation, the microarray slides were removed from their hybridisation chamber using a forceps to gently lift out the insert supporting the slide. The slides were immediately placed in a wash chamber with 110ml of BD GlassHyb Wash solution (BD Biosciences Clontech, UK) and incubated at room temperature on an orbital shaker for 10 minutes. The slides were then washed a further two times in 10ml of BD GlassHyb Wash solution plus 100ml 1X SSC (NaCl, $Na_3Citrate.2H_2O$; pH 7.0) and one final wash in 110ml of 0.1X SSC, each for ten minutes. The slides were briefly rinsed with distilled water and dried by placing in a dry slide container and centrifuged at 1,500g for 5 minutes.

3.2.12 Microarray Scanning

The slides were scanned using an Affymetrix®428™ Array Scanner, a digital confocal laser scanner with an objective lens that scans from above. Fluorescence was measured

after excitation at 532nm and 635nm before conversion into grey-scaled binary data or false-coloured data. A rough preview scan was performed first to determine the area to be scanned and the intensity of the fluorescence. A full scan was then carried out on the slide using normal resolution and a line average of 2. Full scans were performed at a variety of different gain (electronic amplification of signal in image) settings to assess the best signal intensity. Separate raw images for each dye were acquired as 16-bit Tiff files.

3.2.13 Image Analysis

Images were analysed using the software package ImaGene® 5.0 (BioDiscovery, California). The Cy3 and Cy5 image file corresponding to the same slide were loaded directly into ImaGene. The software automatically designated the false colours green and red to the separate images and these images are overlaid on one another. A gene identification file was created in order to identify which spot on the image corresponded to which gene. A file was available from the microarray manufacturer (BD Biosciences Clontech, UK) which needed to be modified into a format compatible with ImaGene. This was a text file with five columns, four of which corressponded to the metarow, metacolumn, row and column where the gene is located on the microarray and the fifth corresponded to the gene name. Once created and saved, the file was loaded into the ImaGene software. The scanned images were upside down to the layout of the gene identification file; thus, it was necessary to rotate both the Cy3 and Cy5 images 180° before analysis could begin.

Following this, a grid template was created in order to identify for the software the location and size of each spot. A subgrid was first created by measuring the average diameter of a spot then entering the number of rows and columns of spots that existed in each block. In the microarrays used, each block had 9 rows and 9 columns, and there were 4 blocks in a metarow and 12 blocks in a metacolumn. Once the subgrid was modified to fit perfectly over all spots in the block, a metagrid made up of 48 blocks was created. This was adjusted manually and using the autoadjust spot function in the software to fit perfectly over all the spots on the microarray image. The grid template was stored as a file and used for all other slides being analysed, with slight adjustments made for each slide.

Segmentation, the differentiation of spot signal and background values within the microarray image, was performed. The percentage of low and high intensity pixels measured was 10% and 90% respectively, for both signal and background. Background buffer, the distance in pixels between the signal and background measurement regions, was set at 5 pixels. Within this region all pixel values are ignored during quantification. Background width, the measurement in pixels to determine how far the background measurements will extend from the buffer region, was also set at 5 pixels.

Quality control measures were performed on all spots to identify badly hybridised spots which could distort the results. Measures were optimised and found to give the best results with the following settings: empty spots were flagged with a threshold of 1.52 ([signal mean – background mean]/ background standard deviation); poor spots were flagged with a threshold of 0.4 (based on morphometry of spots); all negative spots (signal mean< background mean) were flagged. The full microarray image was inspected also and spots manually flagged if deemed unsuitable for analysis.

All spot measurements available were selected for quantification. These were: signal mean, background mean, signal median, background median, signal mode, background mode, signal area, background area, signal total, background total, signal standard deviation, background standard deviation, shape regularity and diameter. The results of the quantification, given as a separate text file for Cy3 and Cy5, were saved as raw data.

3.2.14 Normalisation of raw data

The raw data from image analysis was normalised using the free software ArrayNorm available at http://genome.tugraz.at (Graz University of Technology Bioinformatics group, Austria). The Cy3 and Cy5 files for each microarray slide were loaded into the software and arranged as either Cy3/Cy5 or Cy5/Cy3 ratios, as appropriate. Each experiment was loaded as a different class with the technical replicates loaded as a pair in the class. All the data was subjected to a background subtraction followed by a Lowess normalisation performed separately on each block (subgrid) on each microarray. The Lowess was performed on each subgrid rather than the microarray

slide as a whole because each subgrid is printed with a different tip during manufacture, thus slight differences between each print-tip group occur. Also, the intensity in fluorescence can vary from one end of the slide to the other, so local normalisation is more appropriate. The normalisation transformations were viewed as scatter plots with each individual gene plotted and as box-plots with each subgrid (print-tip group) plotted. Once normalised, the technical replicates in each class were averaged and the data saved as a text file.

3.2.15 Gene expression analysis

The normalised data was analysed using the software package GeneSpring® 7 (Silicon Genetics, California). The text files from normalisation of each of the five experiments contained three columns of information: gene name, GenBank Accession number, and ratio (average). These were used to create a new genome in the software, where the genome is the term given to a set of genes on a microarray. A new parameter was added to the experiment interpretations, called biological replicates. Each experiment was then given the same name under this parameter. This allowed the data to be displayed as either five separate experiments or one set of data, averaged across all five experiments. The data was analysed using log of ratio and the cross gene error model was turned off.

3.2.15.1 Examination of significantly expressed genes

The data was filtered on confidence using the t-test. A p-value of 0.05 was considered significant.

3.2.15.2 Functional Analysis of genes

1. The GeneSpider function in GeneSpring was selected to annotate all information regarding each gene. Using the GenBank Accession numbers, the program retrieved gene information from the Silicon Genetics Mirror Database. Gene Ontology information on the Silicon Genetics Mirror server is obtained from the LocusLink database and is organised into biological processes, molecular functions, and cellular components.

In order to ascertain what the main Gene Ontology (GO) categories were in a particular gene list, a scripted program- SG3c1- from the Bioscript Library in GeneSpring was run.
This program detailed how many genes were in which GO category and which GO categories contained a statistically significant (calculated using a standard Fisher's exact test) number of overlapping genes with the gene list of interest.

2. Using the GO information produced for each gene, all genes were searched for functions relating to cytokines and their regulation.

3. Using the annotated information obtained from GeneSpider, the chromosomal location of each gene could be observed. Genes were then sorted on the basis of their locus. Gene lists were created for loci of particular interest to coeliac disease.

4. The function of genes was further assessed by determining the interaction of the genes in a given biological system or, pathway. Human biological pathways were ftp-downloaded into GeneSpring from a public database called KEGG (Kyoto Encyclopedia of Genes and Genomes), available at ftp://ftp.genome.ad.jp/pub/kegg/pathways. Using the gene lists generated from the above three functional analyses, the pathways in which two or more genes participated were examined.

3.2.15.3 Cluster analysis

Clustering analysis was performed in order to divide genes into groups that have similar expression patterns. Different methods of clustering exist and the following methods were performed and assessed: Hierarchical (Gene tree), K-means and QT Clustering. Hierarchical clustering organises genes into a mock-phylogenetic tree, termed a gene tree, where the location of a branch containing a number of genes is considered a measure of how similar the gene expression patterns are. QT clustering looks for clusters of genes such that each gene in the cluster is within a user-defined distance of every other gene in the cluster, where the cutoff is specified based on a correlation function. K-means clustering divides genes into a user-defined number (k)

of groups based on their expression patterns, producing groups of genes with a high degree of similarity within each group and a low degree of similarity between groups.

A scripted program entitled best k-means was performed which creates different k-means classifications with increasing number of clusters and selects the best classification. Both k-means clustering and QT clustering were assessed by examining the radii of the clusters produced, where the smaller the radius the more tightly grouped and thus more similar the clusters were. Also, the percent explained variability of each clustering method was examined. This number is a measure of the quality of the classification; classifications in which the average radius of each class is small and in which the centroids of each class are located far apart from one another explain a high percentage of the total variability.

Upon selection of the best quality clusters, the genes residing in the clusters were analysed for common transcription factor binding sites. Firstly, the promoter regions (1,500 bp upstream of the predicted transcriptional start site) for genes were identified and retrieved using the Genome Browser (http://genome.ucsc.edu). The promoter regions for all genes in one cluster were loaded into the transcription factor binding site (TFBS) analysis tool, MatInspector (http://www.genomatix.de). This software searches each promoter region separately and then lists the TFBSs that are in common in all the promoters. This search was repeated for each cluster such that a list of TFBSs occurring in each cluster was created. The cluster lists were then filtered against each other so that only TFBSs occurring uniquely in one cluster remained.

3.3 Results

3.3.1 Cell counts

Viable cells were counted before magnetic separation to determine optimum amount of magnetic beads to add. The total cell median value for the coeliac patients was 2.1 x 10^6 and for the control patients was 2.4 x 10^6.

After magnetic depletion of the T-cells, the two fractions of cells were counted. The epithelial cell fraction median value for the coeliac patients was 0.8 x 10^6 and for the control patients was 1.8 x 10^6. The $CD3^+$ T-cell fraction from the coeliac patients had a median value of 0.13 x 10^6 while the control patients had a median value of 0.31 x 10^6.

3.3.2 Purity of epithelial cells

Flow cytometry was performed on an aliquot of the epithelial cell fraction to determine the purity of the cells and ensure optimum depletion of T-cells from this fraction. Ber-EP4 was used as a marker of epithelial cells while CD3 was used to mark any T-cells present. The purity of the epithelial cells was found to be very high (Fig. 3.3.1).

3.3.3 RNA extraction

Gel electrophoresis demonstrated the RNA was free from DNA contamination and not denatured (Fig.3.3.2). Spectrophotometric analysis of the total RNA showed a 260:280nm ratio within the required range of 1.8 to 2.0, which is indicative of RNA free from contamination with protein. Absorbance readings at 260nm were used to determine the quantity of total RNA. The median amount of RNA obtained from the coeliac samples was 2.44μg and from the control samples was 3.28μg.

3.3.4 Amplification of cDNA

The optimum number of PCR cycles was determined for each sample, such that the reaction was completed while the amplification process was still in the exponential phase. An example of the gel photograph taken to determine this number of cycles is given in Fig. 3.3.3. In this sample, the optimum number was determined as 20. The optimised, completed reaction was analysed by electrophoresis to ensure the reaction was successful (Fig.3.3.4). The median yield of purified PCR product was 2.46μg. The

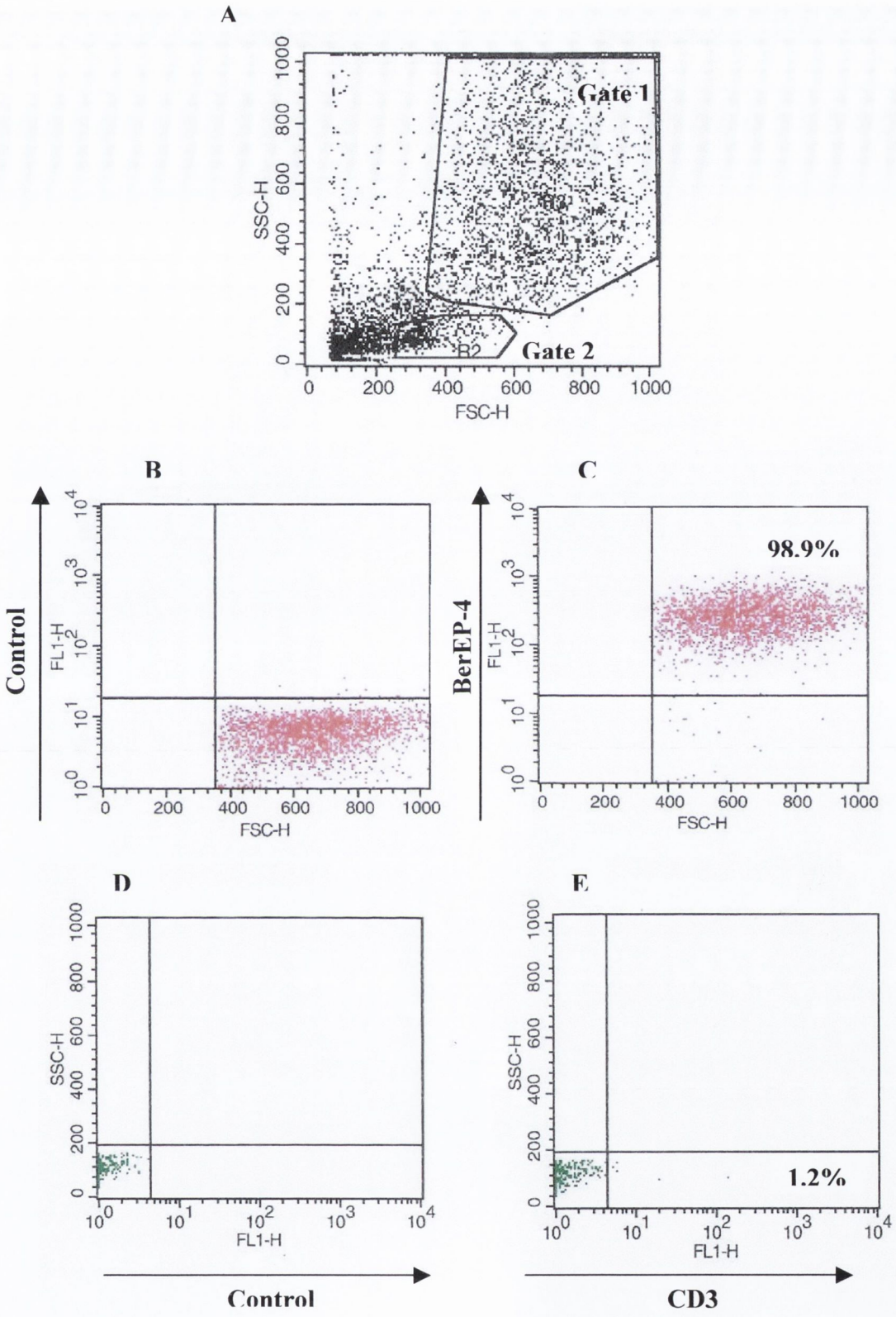
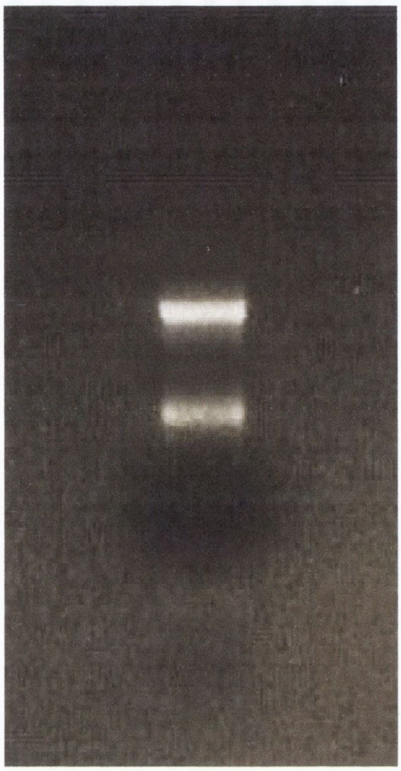


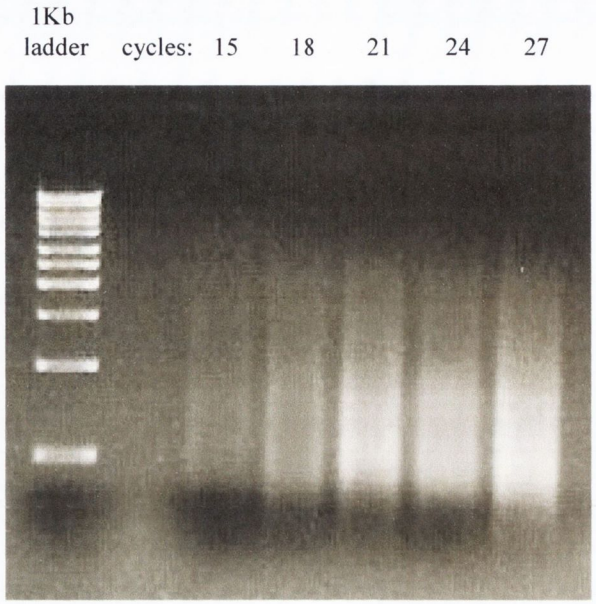
Figure 3.3.1: Flow cytometric analysis of purified epithelial cells. A) Size versus granularity plot. **B)** and **C)** correspond to the population of cells within gate1; and demonstrate control antibody and BerEP-4 expression, respectively. **D)** and **E)** correspond to the population of cells within gate 2; and demonstrate control antibody and CD3 expression, respectively.

Figure 3.3.2: Example of RNA quality



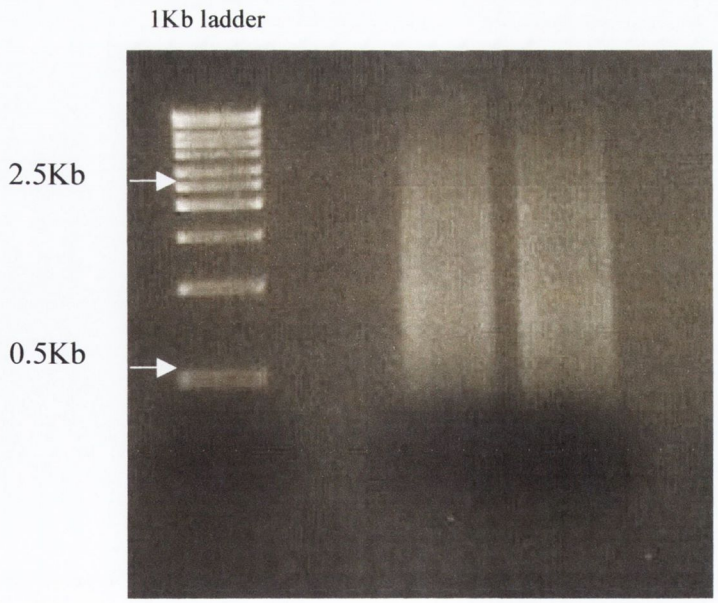
Electrophoresed total RNA (0.5μG) showing bands at 28S and 18S.
The intensity of 28S is greater than that of 18S.

Figure 3.3.3: Optimisation of amplification process.



The plateau phase is deemed to begin at cycle number 21 as all the cDNA smears denoting a cycle number after this point show similar intensity. cDNA smears denoting cycle numbers 15 and 18 show the reaction was still in the exponential phase as their intensity was still increasing. Therefore, in this sample, the optimum cycle number was determined as 20 (one cycle less than 21).

Figure 3.3.4: Completed amplification of cDNA



The sample in this figure corresponds to the sample optimised in Fig.3.3.3. The completed reaction was carried out twice on the same sample. The long smear of cDNA indicates the cDNA is of good quality.

purity of the PCR product from contaminating protein was assessed and a 260:280nm ratio of between 1.80 and 1.86 was found for all samples.

3.3.5 Analysis of labelled probe

The quality and quantity of labelled probe was assessed by UV/VIS spectrophotometry. cDNA yield was determined at 260nm and a median value of 8.7μg was obtained. The baseline values were calculated as described in table 3.2.1 and were all found to be minimal. The Cy3 absorbance was read at 550nm and the median value for all Cy3-labelled probes was found to be 0.05. The Cy5 absorbance was read at 650nm and the median value for Cy5-labelled probes was found to be 0.09. These values were used to calculate the optimal volume of probe (V_{opt}) to hybridise to the microarray using the formula detailed in section 3.2.10.

3.3.6 Microarray scanning

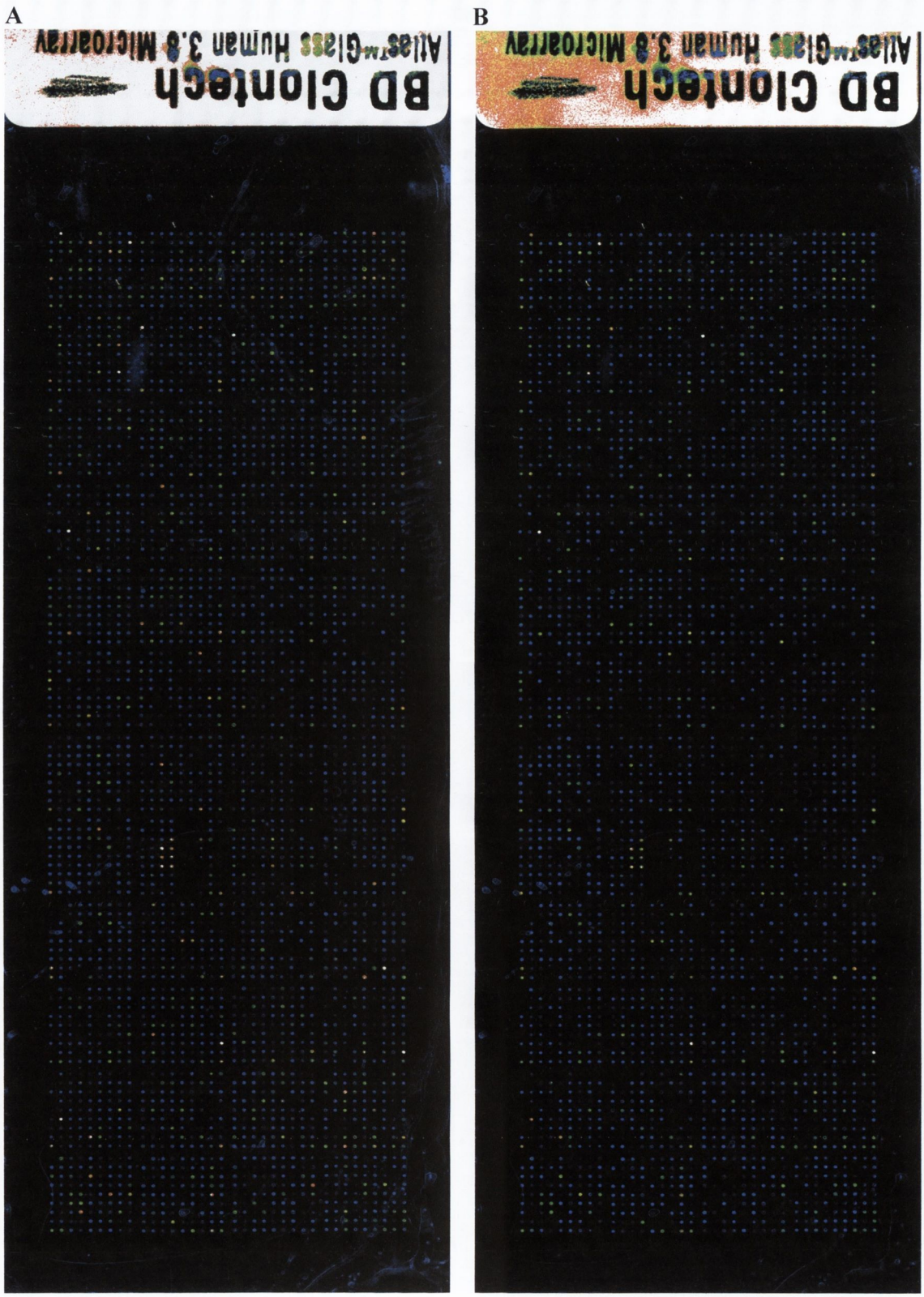
Slides were scanned at different gain settings to determine the optimum signal intensity. Images were selected on the basis of the best compromise between saturation of pixels and low resolution of pixels. Cy3 fluorescence was found to give the best image at a gain of 50 decibels (dB), whereas Cy5 fluorescence gave the best image at a gain of 55dB (Fig. 3.3.5).

3.3.7 Image analysis

Image analysis was performed using the software ImaGene® 5.0 (BioDiscovery, California). Once the slide image was rotated and the gene identification file loaded, each individual spot representing a gene could be inspected. Block (subgrid) E3 was devised by the manufacturers as a control block with a series of positive and negative controls as well as housekeeping genes, to aid in analysis. An example of this block is shown in Fig. 3.3.6. As in Fig. 3.3.6, all slides showed the correct control spots.

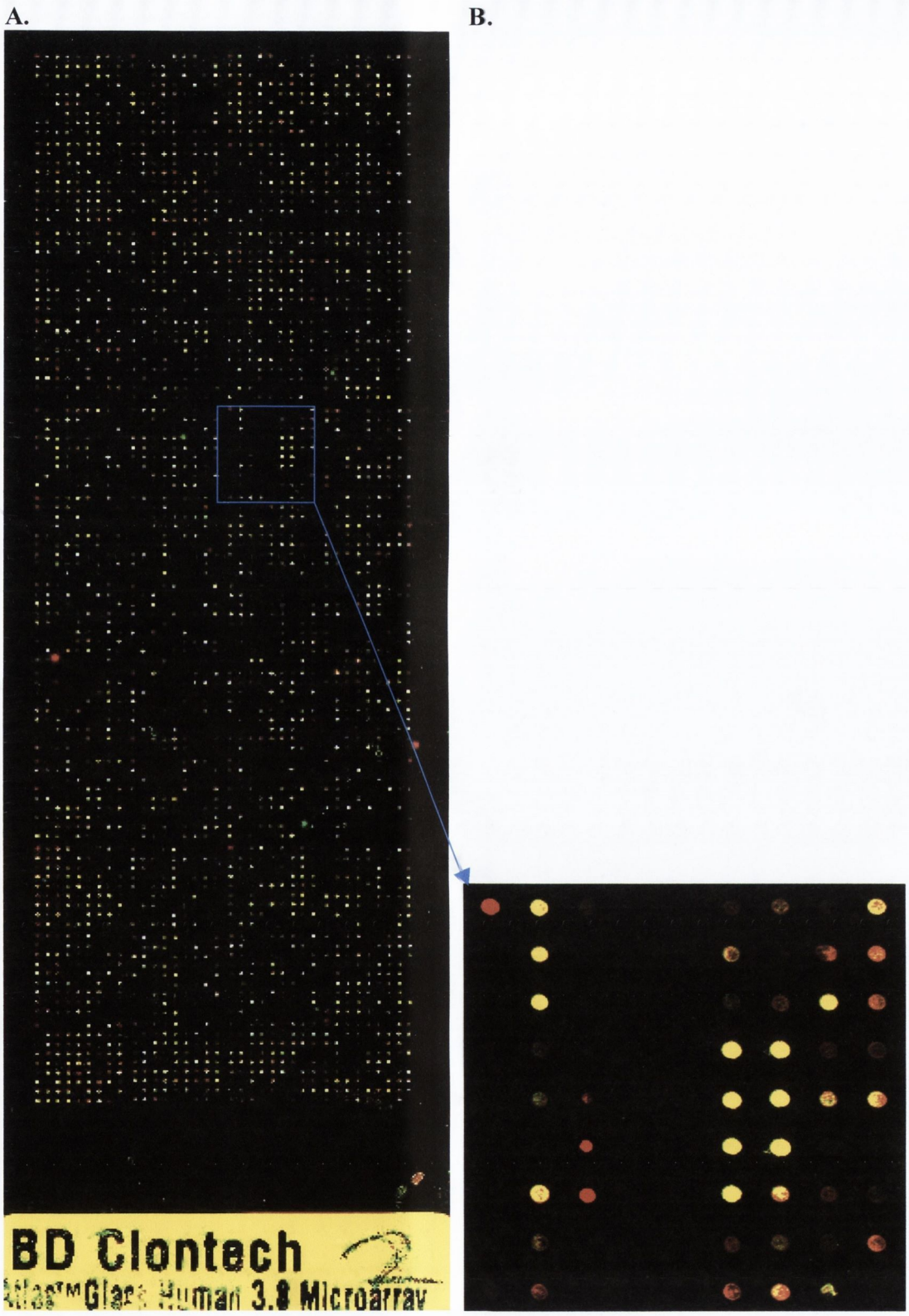
A raw data file listing the quantification of the different measurements was created for each dye. Thus each slide had two files, and ten slides (five pairs) in total were analysed.

Fig. 3.3.5: Scanned images of microarray slides



Images taken of Expt5 slide 1; A= Cy3 (scanned at 532nm) and B= Cy5 (scanned at 635nm).

Fig. 3.3.6: Microarray image

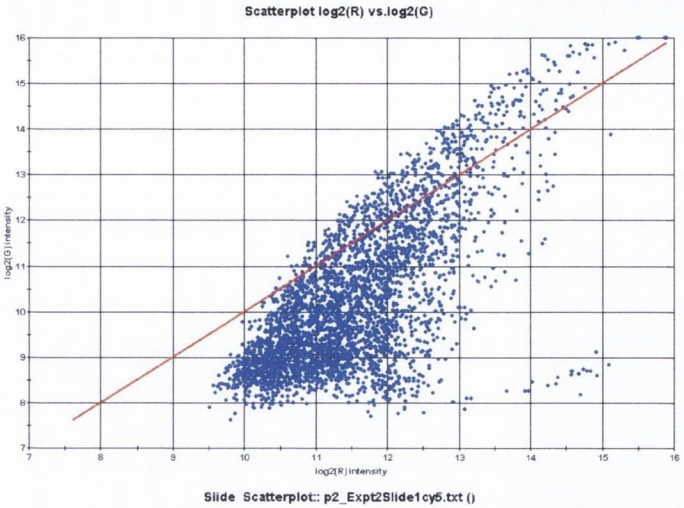


A. Image showing Cy3 and Cy5 images overlaid and whole image rotated. B. Enlarged block E3 containing housekeeping genes.

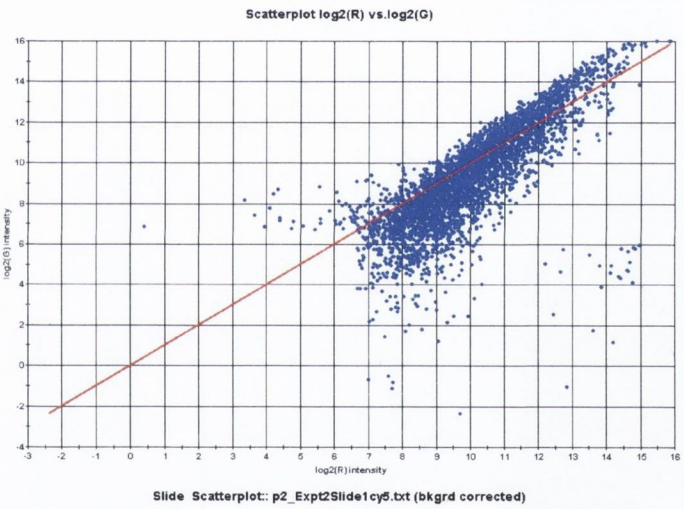
3.3.8 Normalisation

Normalisation was performed on the raw data using the publicly available software, ArrayNorm (http://genome.tugraz.at Graz University of Technology Bioinformatics group, Austria) (Fig.3.3.7). Background fluorescence on the slides was normalised by subtracting the quantified background values from the quantified signal values. The locally-weighted scatterplot smoothing (Lowess) normalisation was used to correct for a bias in fluorescence intensity between the two dyes used. Replicate slide pairs were averaged and the resulting data used for gene expression analysis.

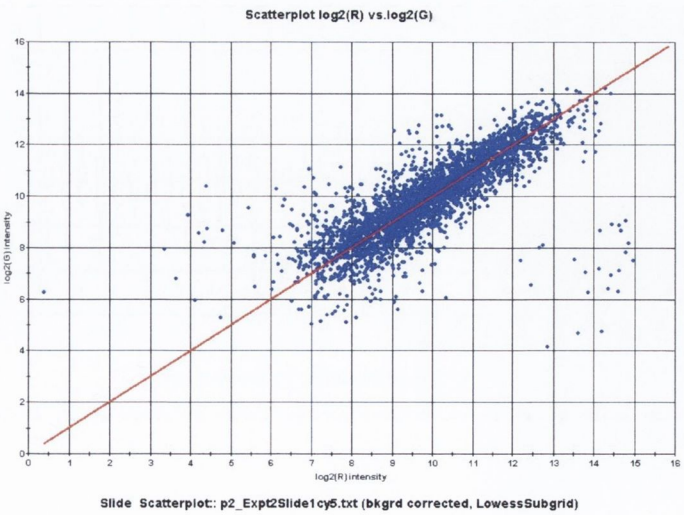
Figure 3.3.7a: Scatterplots showing normalisation of data



Raw data for one slide, each dot representing one gene, plotted Cy3 versus Cy5.

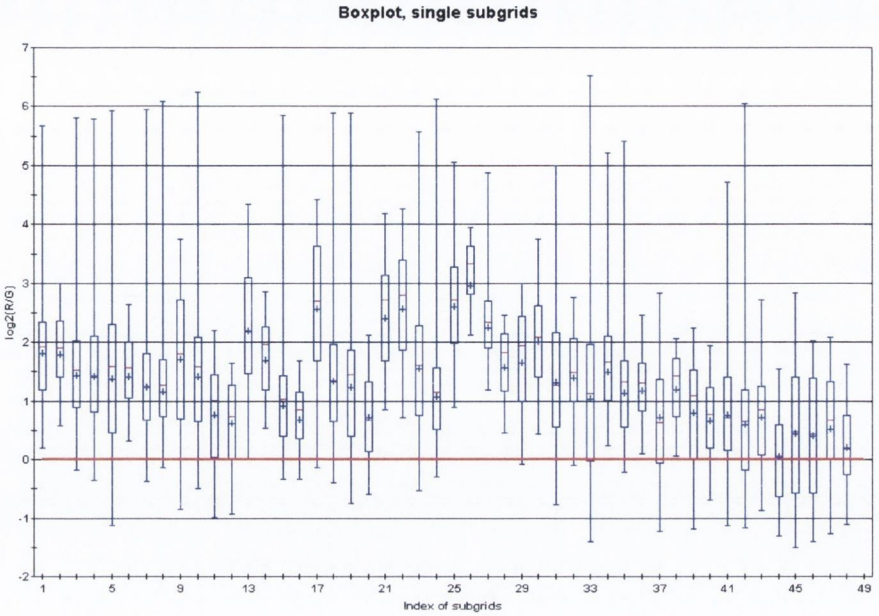


Data with local background value subtracted from signal value.

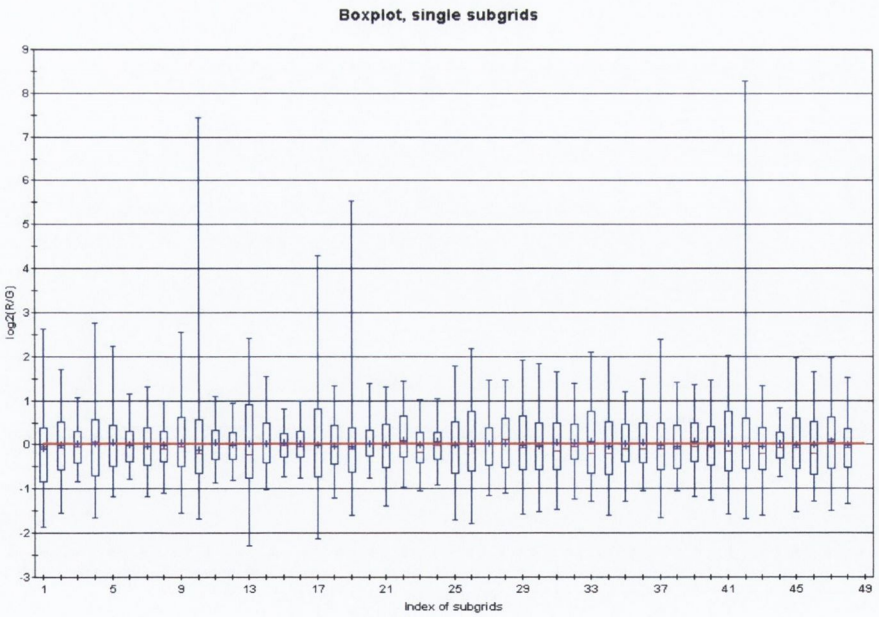


Data with local background value subtracted from signal value and lowess transformation applied.

Figure 3.3.7b: Boxplots showing normalisation of data



Raw data for one slide, each boxplot representing one subgrid



Normalised data, after background correction and Lowess transformation

3.3.9 Gene expression analysis

Gene expression analysis was accomplished using the GeneSpring® 7 software (Silicon Genetics, California). The data was visualised as five separate experiments and as one set of replicated data, displayed as log of ratio (Fig. 3.3.8) (Full list of gene expression results are displayed in Appendix III).

3.3.9.1 Significantly expressed genes

From the 3,800 genes present on the microarrays, 3549 had sufficient data across the five experiments for comparison. Many of these genes showed fold-change ratios with little or no deviation from 1; thus, to focus on only differentially expressed genes, the list was filtered on a fold-change of 1.25-fold. A fold-change of 1.25 has been described in previously published microarray experiments [Naef *et al.* 2002; Juuti-Uusitalo *et al.* 2004] and has been shown to indicate reliable differneces in gene expresssion [Naef *et al.* 2002]. This filtering yielded a list of 1,256 genes with which to perform analysis. Of these 1,256 genes, 827 were up-regulated and 429 were down-regulated. A p-value of 0.05 was used as a cut-off to distinguish significantly expressed genes which yielded a gene list of 102 genes which are displayed in table 3.3.1.

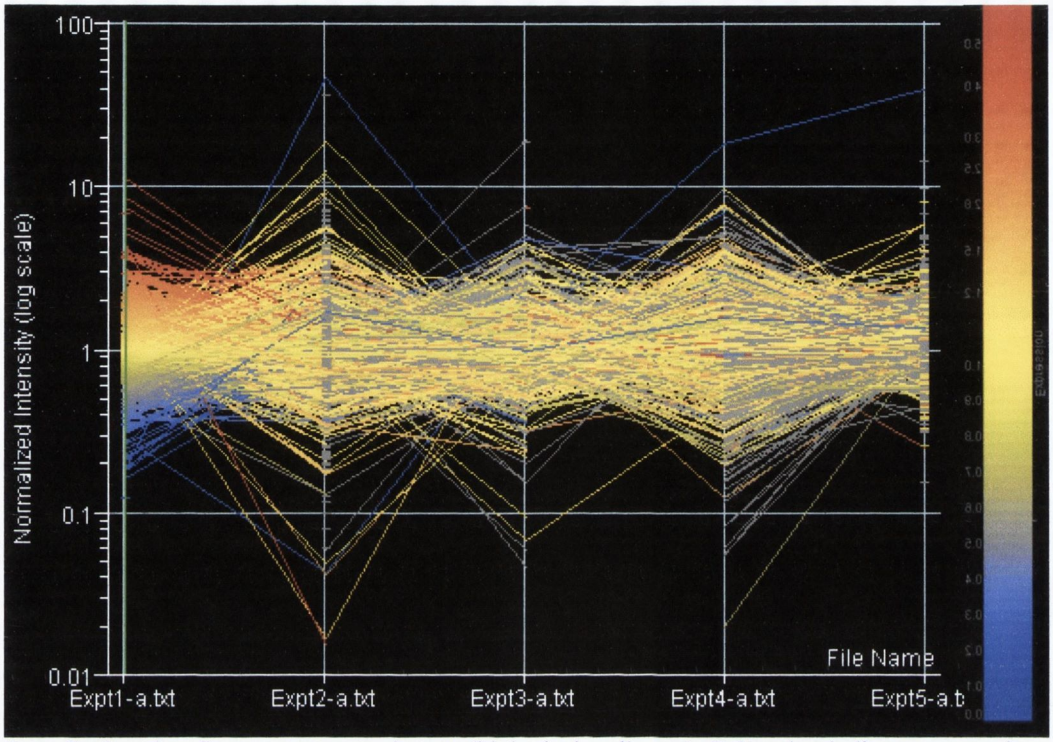
3.3.9.2 Functional analysis of genes

– A scripted program was run to find the most important gene ontology (GO) functions in the gene list of 102 genes. A GO function was deemed over-represented amongst the gene list if it was found to have a statistically significant number of genes in common with the gene list. These GO functions are represented as a pie-chart in Figure 3.3.9. The top three functions, cell growth and/or maintenance, metabolism and development are categories of biological processes while the remaining 14 functions are sub-categories within the first three functions.

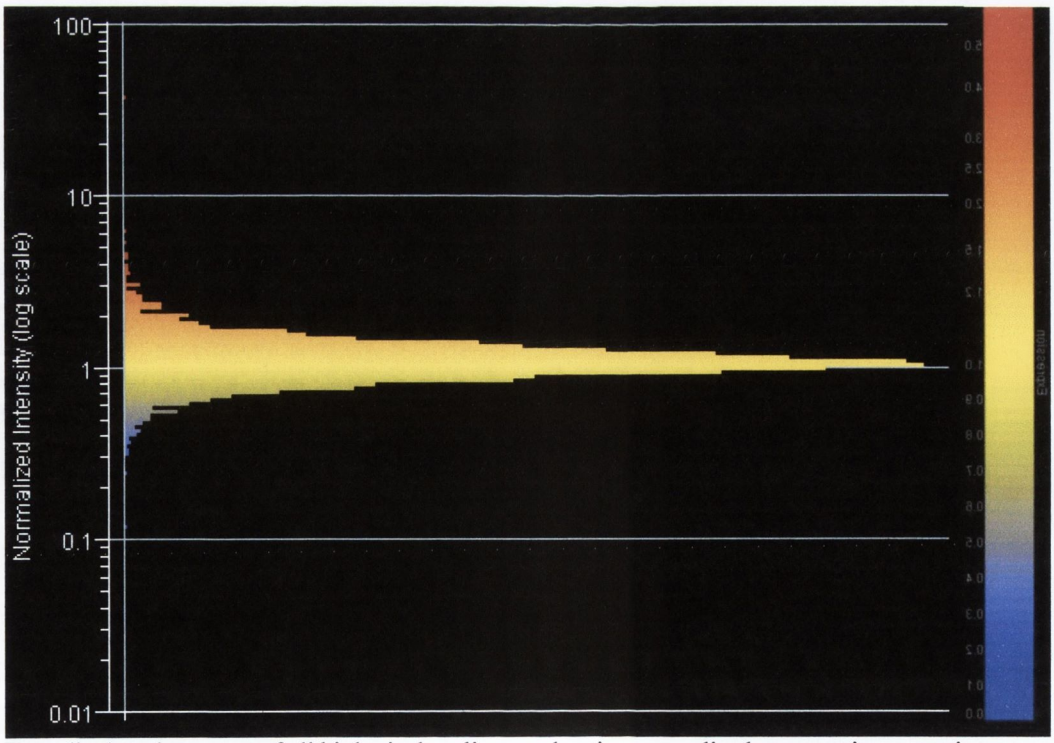
– As cytokines and their regulation are a key functional area in coeliac disease, a list was constructed containing genes for cytokines, cytokine receptors and genes affecting and affected by cytokines. There were 60 such genes on the microarray, of which 23 showed expression changes in the same direction in all experiments (Table 3.3.2).

- All genes were sorted on the basis of their chromosomal location. The most strongly implicated loci are Chromosome 6p21, known as CELIAC1 locus, Chromosome 5q31-

Figure 3.3.8: Normalised gene expression data



Data displayed as five separate experiments (biological replicates). Colour-bar indicates expression levels where red is over-expressed and blue under-expressed.



Data displayed as mean of all biological replicates, showing normalised parametric expression.

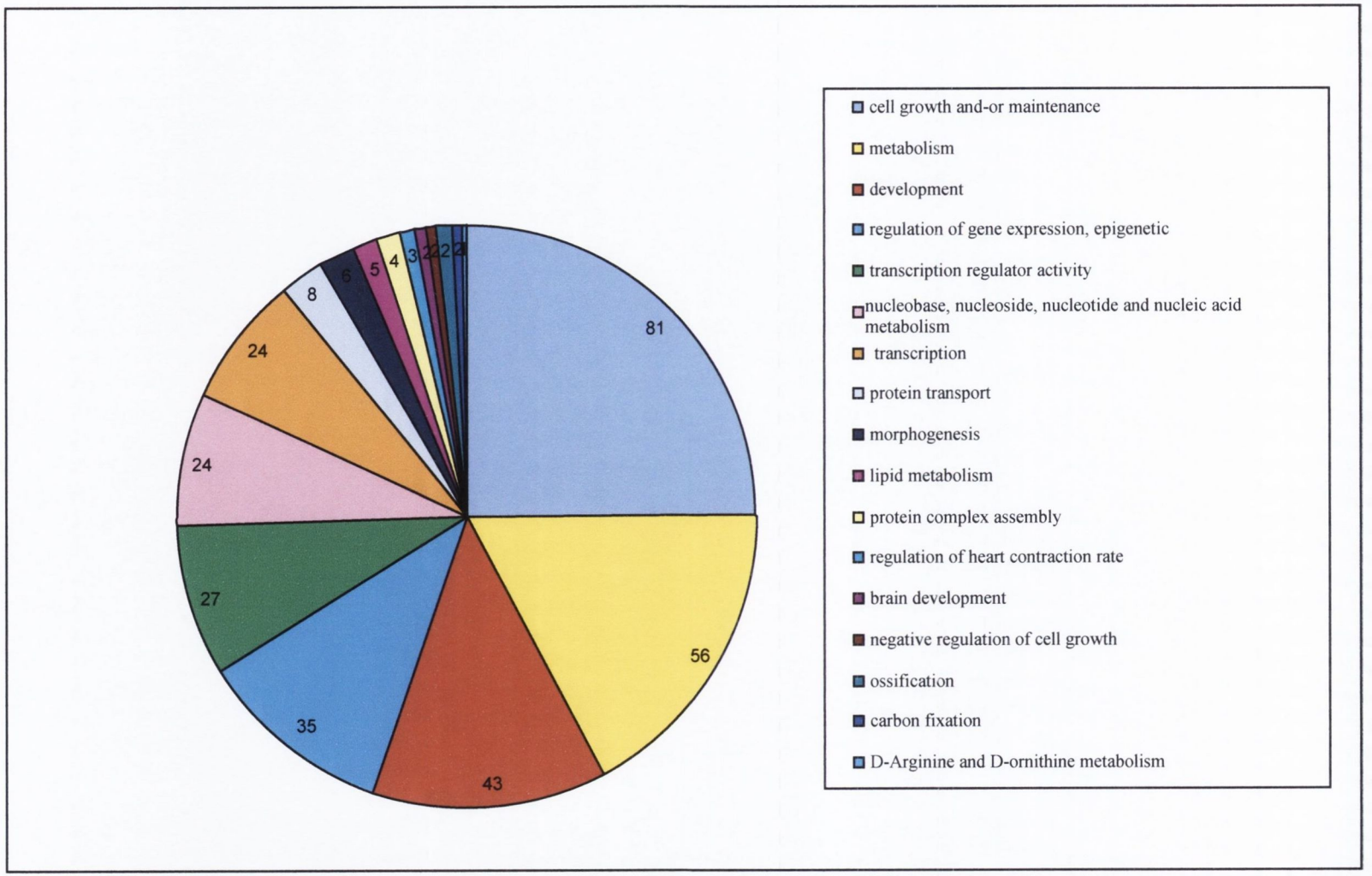
Table 3.3.1: Significantly expressed genes (p<0.05, fold-change > or < 1.25-fold)

Gene Name	GenBank Accession Number	Fold-change	p-value
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	NM_002130	0.535 (0.431 to 0.68)	0.00679
6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	NM_004567	0.648 (0.634 to 0.662)	0.032
A kinase (PRKA) anchor protein 1	NM_005751	0.748 (0.74 to 0.757)	0.0248
adenosine kinase	NM_001123	0.749 (0.581 to 1.017)	0.0406
aminolevulinate, delta-, synthase 1	NM_000688	1.453 (1.444 to 1.461)	0.0105
amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)	NM_001164	2.218 (2.154 to 2.284)	0.0234
AND-1 protein	NM_007086	1.659 (1.598 to 1.721)	0.0465
ash2 (absent, small, or homeotic)-like (Drosophila)	NM_004674	0.749 (0.613 to 0.904)	0.0368
ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A	NM_001691	1.47 (1.116 to 2.178)	0.0364
bone marrow stromal cell antigen 2	NM_004335	1.29 (1.239 to 1.352)	0.00975
BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase)	NM_004656	1.316 (1.306 to 1.327)	0.0185
calcium channel, voltage-dependent, L type, alpha 1C subunit	NM_000719	1.351 (1.343 to 1.358)	0.0112
CD3G antigen, gamma polypeptide (TiT3 complex)	NM_000073	1.547 (1.54 to 1.554)	0.00676
choline kinase	NM_001277	1.507 (1.478 to 1.536)	0.0303
cholinergic receptor, nicotinic, alpha polypeptide 5	NM_000745	0.557 (0.532 to 0.611)	0.00605
chromosome 18 open reading frame 1	NM_004338	1.743 (1.43 to 2.651)	0.0298
chromosome X open reading frame 2	NM_001586	0.762 (0.708 to 0.836)	0.0308
cystatin A (stefin A)	NM_005213	1.48 (1.469 to 1.49)	0.0114
cytoplasmic linker 2	NM_003388	1.309 (1.201 to 1.552)	0.00603
D-amino-acid oxidase	NM_001917	1.475 (1.448 to 1.503)	0.03
developmentally regulated GTP binding protein 1	NM_004147	1.56 (1.554 to 1.566)	0.00551
dihydropyrimidinase	NM_001385	0.638 (0.505 to 0.792)	0.0205
dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3	NM_001396	2.627 (2.508 to 2.751)	0.0304
EGF-containing fibulin-like extracellular matrix protein 1	NM_004105	1.276 (1.179 to 1.525)	0.0273
ELK4, ETS-domain protein (SRF accessory protein 1)	NM_001973	1.446 (1.026 to 1.779)	0.0216
envoplakin	NM_001988	0.69 (0.566 to 0.844)	0.0201
erythropoietin	NM_000799	2.738 (2.533 to 2.961)	0.0492
eukaryotic translation elongation factor 1 beta 2	NM_001959	1.413 (1.41 to 1.417)	0.0051
eukaryotic translation initiation factor 4E binding protein 3	NM_003732	1.466 (1.342 to 1.568)	0.0143
exportin 1 (CRM1 homolog, yeast)	NM_003400	1.847 (1.595 to 2.199)	0.0225
flap structure-specific endonuclease 1	NM_004111	1.288 (1.279 to 1.296)	0.0167
forkhead box G1B	NM_005249	1.558 (1.531 to 1.585)	0.0248
fructose-1,6-bisphosphatase 1	NM_000507	1.535 (1.535 to 1.536)	0.000296

Gene Name	GenBank Accession Number	Fold-change	p-value
gamma-aminobutyric acid (GABA) A receptor, beta 3	NM_000814	1.74 (1.474 to 1.981)	0.00286
gap junction protein, alpha 4, 37kDa (connexin 37)	NM_002060	1.952 (1.94 to 1.964)	0.00575
glucosidase, beta; acid (includes glucosylceramidase)	NM_000157	1.258 (1.258 to 1.259)	0.000369
G protein-coupled receptor 7	NM_005285	2.293 (1.875 to 2.985)	0.0265
glutamate receptor, metabotropic 7	NM_000844	0.768 (0.735 to 0.838)	0.026
GPI anchored molecule like protein	NM_007264	1.791 (1.584 to 1.957)	0.0116
growth arrest-specific 6	NM_000820	1.576 (1.526 to 1.626)	0.0443
growth factor receptor-bound protein 7	NM_005310	1.564 (1.277 to 1.757)	0.0479
hematopoietic cell-specific Lyn substrate 1	NM_005335	1.585 (1.304 to 2.479)	0.0164
hemochromatosis	NM_000410	1.319 (1.313 to 1.324)	0.00924
high-mobility group box 3	NM_002128	1.775 (1.509 to 1.974)	0.02
histatin 1	NM_002159	0.75 (0.615 to 0.847)	0.0256
histone deacetylase 2	NM_001527	1.574 (1.275 to 2.03)	0.0178
homeo box A11	NM_005523	1.325 (1.064 to 1.641)	0.0246
homeo box A7	NM_006896	1.701 (1.171 to 2.783)	0.0297
human T-cell leukemia virus enhancer factor	NM_002158	1.267 (1.252 to 1.282)	0.0311
integrin beta 4 binding protein	NM_002212	2.356 (1.882 to 2.647)	0.0167
lipoprotein, Lp(a)	NM_005577	1.653 (1.211 to 1.908)	0.0174
MAP/microtubule affinity-regulating kinase 2	NM_004954	0.717 (0.644 to 0.759)	0.00307
matrix Gla protein	NM_000900	0.659 (0.655 to 0.662)	0.00909
membrane protein, palmitoylated 3 (MAGUK p55 subfamily member 3)	NM_001932	1.259 (1.254 to 1.264)	0.0109
methyl CpG binding protein 2 (Rett syndrome)	NM_004992	0.532 (0.328 to 0.711)	0.0326
microseminoprotein, beta-	NM_002443	1.592 (1.128 to 2.155)	0.0113
microtubule-associated protein 1A	NM_002373	0.652 (0.549 to 0.729)	0.0395
N-acetylgalactosaminidase, alpha-	NM_000262	0.783 (0.672 to 0.904)	0.0278
NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase)	NM_002496	0.78 (0.668 to 0.872)	0.0242
natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	NM_000906	0.693 (0.576 to 0.863)	0.00936
neuronal PAS domain protein 2	NM_002518	1.288 (1.192 to 1.445)	0.0497
Notch homolog 3 (Drosophila)	NM_000435	1.271 (1.07 to 1.399)	0.0285
nuclear transcription factor Y, alpha	NM_002505	0.714 (0.606 to 0.84)	0.0196
nudix (nucleoside diphosphate linked moiety X)-type motif 2	NM_001161	2.209 (2.11 to 2.313)	0.037
oligodendrocyte myelin glycoprotein	NM_002544	2.008 (1.591 to 2.59)	0.0386
oxidase (cytochrome c) assembly 1-like	NM_005015	0.625 (0.614 to 0.637)	0.0241
paired box gene 8	NM_003466	1.419 (1.396 to 1.441)	0.0289
peroxisome biogenesis factor 13	NM_002618	2.276 (2.267 to 2.286)	0.00331
potassium inwardly-rectifying channel, subfamily J, inhibitor 1	NM_002244	1.219 (1.218 to 1.219)	0.000159
potassium voltage-gated channel, Isk-related family, member 1	NM_000219	1.369 (1.109 to 1.691)	0.00959

Gene Name	GenBank Accession Number	Fold-change	p-value
PRKC, apoptosis, WT1, regulator	NM_002583	0.73 (0.721 to 0.739)	0.0251
procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2	NM_000935	1.563 (1.556 to 1.57)	0.00657
protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)	NM_006223	0.628 (0.595 to 0.685)	0.0086
PTK2B protein tyrosine kinase 2 beta	NM_004103	0.719 (0.692 to 0.738)	0.00362
pyrroline-5-carboxylate reductase 1	NM_006907	1.31 (1.198 to 1.408)	0.0059
prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia)	NM_000311	0.539 (0.48 to 0.577)	0.00875
pyruvate kinase, muscle	NM_002654	1.438 (1.33 to 1.638)	0.031
Ras-like without CAAX 1	NM_006912	1.306 (1.177 to 1.376)	0.0356
retinal G protein coupled receptor	NM_002921	0.744 (0.728 to 0.76)	0.0458
retinol binding protein 1, cellular	NM_002905	0.692 (0.533 to 0.93)	0.0305
retinol dehydrogenase 5 (11-cis and 9-cis)	NM_002900	1.446 (1.319 to 1.54)	0.0158
ribonuclease, RNase A family, 4	NM_002937	1.279 (1.256 to 1.302)	0.0467
ribosomal protein L19	NM_000981	1.744 (1.457 to 2.053)	0.00118
ribosomal protein S29	NM_001032	1.311 (1.28 to 1.367)	0.00579
sialyltransferase 1 (beta-galactoside alpha-2,6-sialyltransferase)	NM_003032	1.547 (1.182 to 1.874)	0.0207
small proline-rich protein 3	NM_005416	2.095 (2.001 to 2.195)	0.0398
sorcin	NM_003130	1.316 (1.218 to 1.601)	0.0248
spectrin, alpha, erythrocytic 1 (elliptocytosis 2)	NM_003126	1.29 (1.284 to 1.296)	0.0108
SRY (sex determining region Y)-box 14	NM_006942	0.732 (0.684 to 0.779)	0.0142
sterol regulatory element binding transcription factor 1	NM_004176	4.529 (4.164 to 4.926)	0.0354
superkiller viralicidic activity 2-like (S. cerevisiae)	NM_006929	1.639 (1.244 to 2.352)	0.0401
syntaxin 3A	NM_004177	1.81 (1.514 to 2.238)	0.00578
TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68kDa	NM_003487	0.656 (0.554 to 0.755)	0.0432
T-box 6	NM_004608	0.73 (0.717 to 0.743)	0.037
transmembrane 7 superfamily member 1 (upregulated in kidney)	NM_003272	0.761 (0.749 to 0.773)	0.0371
tubulin-specific chaperone a	NM_004607	0.725 (0.673 to 0.799)	0.0239
tumor necrosis factor receptor superfamily, member 18	NM_004195	1.941 (1.875 to 2.01)	0.0334
tyrosinase (oculocutaneous albinism IA)	NM_000372	1.272 (1.211 to 1.403)	0.0387
ubiquitin protein ligase E3A (human papilloma virus E6-associated protein, Angelman syndrome)	NM_000462	0.771 (0.762 to 0.78)	0.0286
v-Ha-ras Harvey rat sarcoma viral oncogene homolog	NM_005343	0.631 (0.475 to 0.83)	0.0282
vinculin	NM_003373	1.752 (1.407 to 2.212)	0.00217
wingless-type MMTV integration site family, member 7A	NM_004625	1.33 (1.039 to 1.508)	0.044

Figure 3.3.9: Gene Ontology functions of significantly altered genes



Displayed GO functions contain a statistically significant (using Fisher's exact test) number of overlapping genes with the list of significantly altered genes

Table 3.3.2: Genes involved in cytokine function

Gene Name	Fold-change (range)	p-value
interleukin 3 (colony-stimulating factor, multiple)	2.52 (1.742 to 3.647)	0.242
interleukin 17 (cytotoxic T-lymphocyte-associated serine esterase 8)	1.407 (1 to 1.98)	0.5
interleukin 18 (interferon-gamma-inducing factor)	1.973 (1.15 to 3.995)	0.207
interleukin 11 receptor, alpha	1.181 (1.029 to 1.269)	0.138
interleukin 13 receptor, alpha 1	1.048 (1.001 to 1.097)	0.492
interleukin 18 receptor 1	1.095 (1.003 to 1.195)	0.488
interleukin-1 receptor-associated kinase 1	1.229 (1.002 to 1.736)	0.186
interleukin-1 receptor-associated kinase 2	1.571 (1.267 to 1.949)	0.283
interleukin 6 signal transducer (gp130, oncostatin M receptor)	1.237 (1.216 to 1.258)	0.0509
interferon, alpha 8	1.656 (1.165 to 3.123)	0.253
interferon, alpha 16	1.559 (1.072 to 2.749)	0.264
interferon, alpha-inducible protein 27	3.821 (2.876 to 5.078)	0.133
protein-kinase, interferon-inducible double stranded RNA dependent inhibitor, repressor of (P58 repressor)	0.819 (0.771 to 0.868)	0.0286
myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	1.33 (1.249 to 1.415)	0.137
guanylate binding protein 1, interferon-inducible, 67kDa	1.379 (1.216 to 1.563)	0.237
interferon-induced protein with tetratricopeptide repeats 4	1.14 (1.071 to 1.213)	0.282
interferon-related developmental regulator 1	0.848 (0.741 to 0.97)	0.436
tumor necrosis factor, alpha-induced protein 3	1.2 (1.164 to 1.236)	0.104
tumor necrosis factor (ligand) superfamily, member 9	0.898 (0.897 to 0.899)	0.008
tumor necrosis factor (ligand) superfamily, member 12	1.527 (1.026 to 3.149)	0.363
tumor necrosis factor (ligand) superfamily, member 13	1.292 (1.14 to 1.464)	0.289
tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	1.603 (1.113 to 2.309)	0.419
tumor necrosis factor receptor superfamily, member 18	1.941 (1.875 to 2.01)	0.0334

Table 3.3.3a: Genes found in CELIAC1 locus (chromosome 6p21)

Gene Name	GenBank Accesion Number	chromo-somal location	Mean fold-change (Range)	p-value
hemochromatosis	NM_000410	6p21.3	1.319 (1.313 to 1.324)	0.00924
nuclear transcription factor Y, alpha	NM_002505	6p21.3	0.714 (0.606 to 0.84)	0.0196
superkiller viralicidic activity 2-like (S. cerevisiae)	NM_006929	6p21	1.639 (1.244 to 2.352)	0.0401
major histocompatibility complex, class II, DR beta 1	NM_002124	6p21.3	0.929 (0.924 to 0.934)	0.0439
small nuclear ribonucleoprotein polypeptide C	NM_003093	6p21.31	1.167 (1.15 to 1.185)	0.0598
major histocompatibility complex, class II, DM alpha	NM_006120	6p21.3	1.226 (1.197 to 1.256)	0.0745
histone 1, H1c	NM_005319	6p21.3	1.414 (1.108 to 1.861)	0.0776
nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1	NM_005007	6p21.3	1.321 (1.144 to 1.827)	0.0867
glial cells missing homolog 1 (Drosophila)	NM_003643	6p21-p12	1.417 (1.006 to 1.982)	0.0897
suppressor of Ty 3 homolog (S. cerevisiae)	NM_003599	6p21.1-p21.3	1.329 (1.021 to 1.532)	0.164
major histocompatibility complex, class II, DP beta 1	NM_002121	6p21.3	1.871 (1.321 to 3.36)	0.167
protein phosphatase 2, regulatory subunit B (B56), delta isoform	NM_006245	6p21.1	0.411 (0.319 to 0.53)	0.177
histone 1, H4a	NM_003538	6p21.3	1.097 (1.065 to 1.129)	0.195
transcription factor 19 (SC1)	NM_007109	6p21.3	1.08 (1.049 to 1.112)	0.231
proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional protease 7)	NM_004159	6p21.3	1.509 (1.287 to 1.77)	0.235
RD RNA binding protein	NM_002904	6p21.3	0.623 (0.351 to 0.881)	0.243
MHC class II HLA DR5 DR-beta-chain	NM_002125	6p21.3	0.375 (0.0493 to 0.993)	0.248
histone 1, H4f	NM_003540	6p21.3	1.221 (1.06 to 1.578)	0.261
kelch domain containing 3	NM_057161	6p21.1	1.103 (1 to 1.246)	0.269
BCL2-antagonist/killer 1	NM_001188	6p21.3	0.784 (0.697 to 0.881)	0.285
guanylate cyclase activator 2A (guanylin)	NM_002098	6p21.1	1.346 (1.159 to 1.563)	0.297
transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	NM_000544	6p21.3	0.501 (0.177 to 0.869)	0.316
B-factor, properdin	NM_001710	6p21.3	0.885 (0.821 to 0.954)	0.35
zinc finger protein 76 (expressed in testis)	NM_003427	6p21.3-p21.2	1.43 (1.124 to 1.819)	0.377
splicing factor, arginine/serine-rich 3	NM_003017	6p21	1.08 (1.024 to 1.138)	0.383
RIO kinase 3 (yeast)	NM_006913	6p21.3	1.236 (1.029 to 1.484)	0.453
guanine nucleotide binding protein-like 1	NM_005275	6p21.3	1.072 (1.007 to 1.142)	0.467

Table 3.3.3b: Genes found in CELIAC2 locus (chromosome 5q31-33)

Gene Name	GenBank Accesion Number	Chromo-somal location	Mean fold-change (range)	p-value
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8kDa	NM_002488	5q31	0.905 (0.899 to 0.912)	0.0486
IL2-inducible T-cell kinase	NM_005546	5q31-q32	1.207 (1.114 to 1.408)	0.134
glycine receptor, alpha 1 (startle disease/hyperekplexia, stiff man syndrome)	NM_000171	5q32	0.633 (0.419 to 0.826)	0.16
MAD, mothers against decapentaplegic homolog 5 (Drosophila)	NM_005903	5q31	1.183 (1.126 to 1.242)	0.18
histidyl-tRNA synthetase	NM_002109	5q31.3	0.828 (0.695 to 0.988)	0.205
non-metastatic cells 5, protein expressed in (nucleoside-diphosphate kinase)	NM_003551	5q31	1.17 (1.104 to 1.24)	0.225
interleukin 3 (colony-stimulating factor, multiple)	NM_000588	5q31.1	2.52 (1.742 to 3.647)	0.242
prefoldin 1	NM_002622	5q31	1.091 (1.052 to 1.133)	0.256
paired-like homeodomain transcription factor 1	NM_002653	5q31	2.267 (1.566 to 3.282)	0.27
sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican)	NM_004598	5q31	0.813 (0.734 to 0.899)	0.289
serine protease inhibitor, Kazal type 1	NM_003122	5q32	1.166 (1.029 to 1.321)	0.435

Table 3.3.3c: Genes found in CELIAC3 locus (chromosome 2q33)

Gene Name	GenBank Accession Number	Chromo-somal location	Mean fold-change	p-value
chemokine (C-C motif) ligand 20	NM_004591	2q33-q37	0.821 (0.802 to 0.841)	0.0751
crystallin, gamma B	NM_005210	2q33-q35	1.526 (1.189 to 1.811)	0.0802
parathyroid hormone receptor 2	NM_005048	2q33	0.728 (0.696 to 0.761)	0.0889
heat shock 10kDa protein 1 (chaperonin 10)	NM_002157	2q33.1	1.292 (1.209 to 1.379)	0.16
DnaJ (Hsp40) homolog, subfamily B, member 2	NM_006736	2q32-q34	1.272 (1.025 to 1.498)	0.166
frizzled homolog 7 (Drosophila)	NM_003507	2q33	0.836 (0.536 to 0.99)	0.189
a disintegrin and metalloproteinase domain 23	NM_003812	2q33	0.951 (0.922 to 0.98)	0.347
eukaryotic translation elongation factor 1 beta 2	NM_001959	2q33-q34	1.123 (1.044 to 1.209)	0.359
caspase 8, apoptosis-related cysteine protease	NM_001228	2q33-q34	0.762 (0.639 to 0.908)	0.364
BCS1-like (yeast)	NM_004328	2q33	0.52 (0.312 to 0.866)	0.422

Table 3.3.3d: Genes found in CELIAC4 locus (chromosome 19p13)

Gene Name	GenBank Accession Number	Chromo-somal location	Mean fold-change (range)	p-value
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa	NM_004146	19p13.12-p13.11	1.295 (1.246 to 1.345)	0.0938
regulatory factor X, 1 (influences HLA class II expression)	NM_002918	19p13.1	0.86 (0.833 to 0.888)	0.135
myosin IXB	NM_004145	19p13.1	1.104 (1.073 to 1.136)	0.177
solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6	NM_005071	19p13.12	1.339 (1.218 to 1.472)	0.2
syntaxin 10	NM_003765	19p13.13	0.764 (0.7 to 0.834)	0.2
phosphodiesterase 4C, cAMP-specific (phosphodiesterase E1 dunce homolog, Drosophila)	NM_000923	19p13.11	1.536 (1.313 to 1.797)	0.223
prostaglandin E receptor 1 (subtype EP1), 42kDa	NM_000955	19p13.1	0.905 (0.868 to 0.944)	0.252
zinc finger protein 91 (HPF7, HTF10)	NM_003430	19p13.1-p12	2.209 (1.33 to 3.668)	0.362
zinc finger protein 43 (HTF6)	NM_003423	19p13.1-p12	0.598 (0.408 to 0.876)	0.407

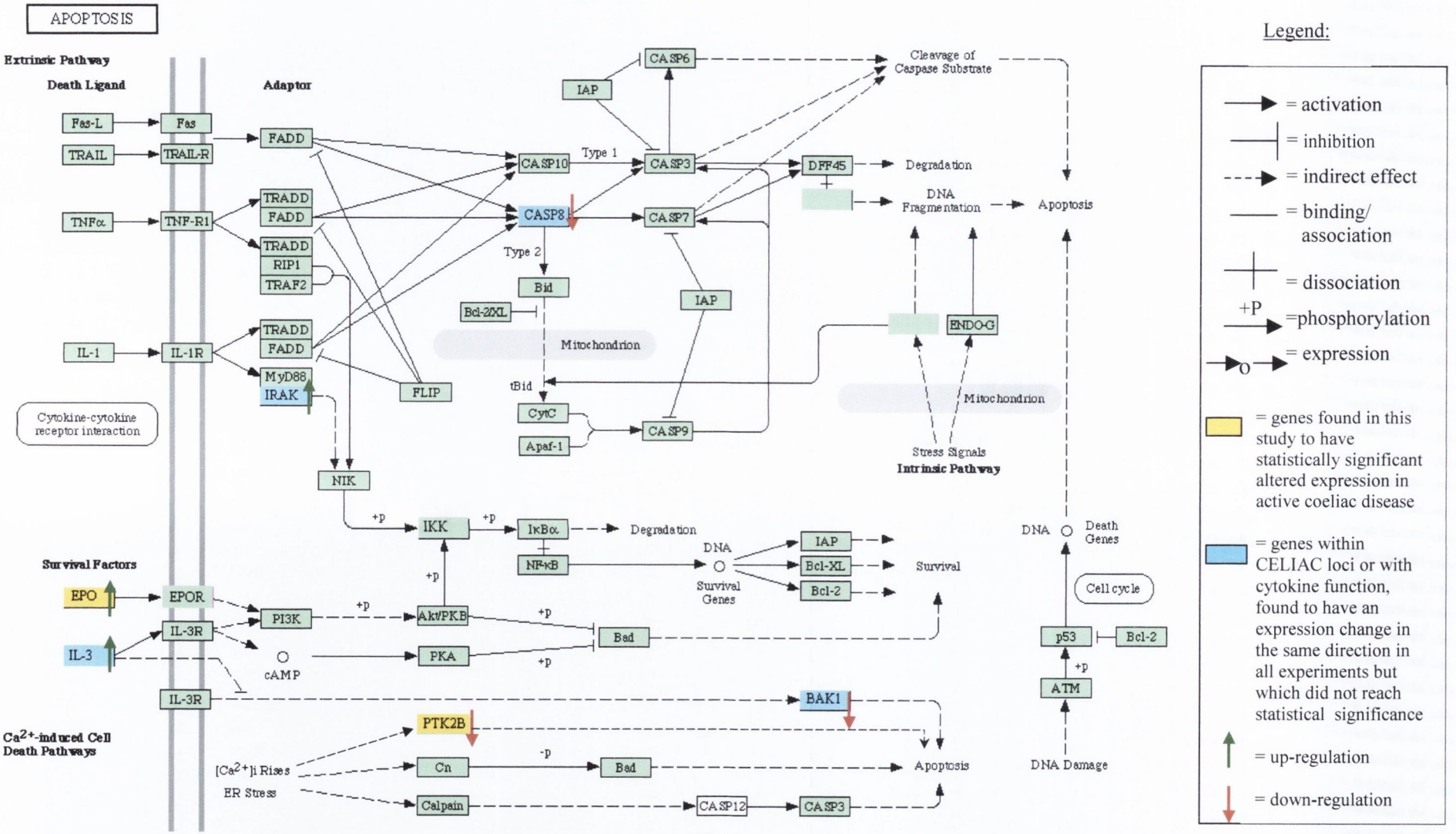


Figure 3.3.10a: Apoptosis pathway showing involvement of genes with altered expression in active coeliac disease

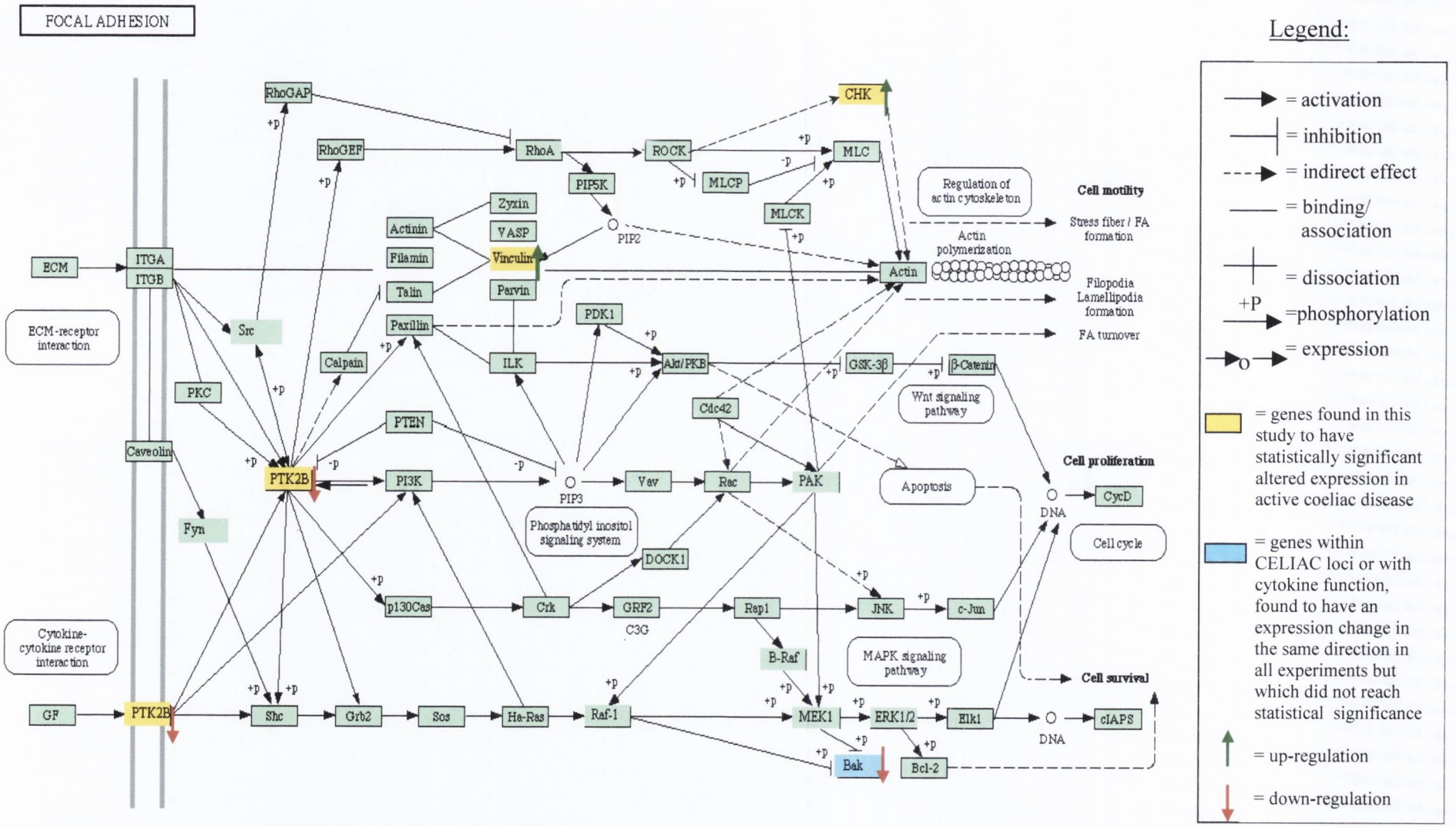


Figure 3.3.10b: Focal adhesion pathway showing involvement of genes with altered expression in active coeliac disease

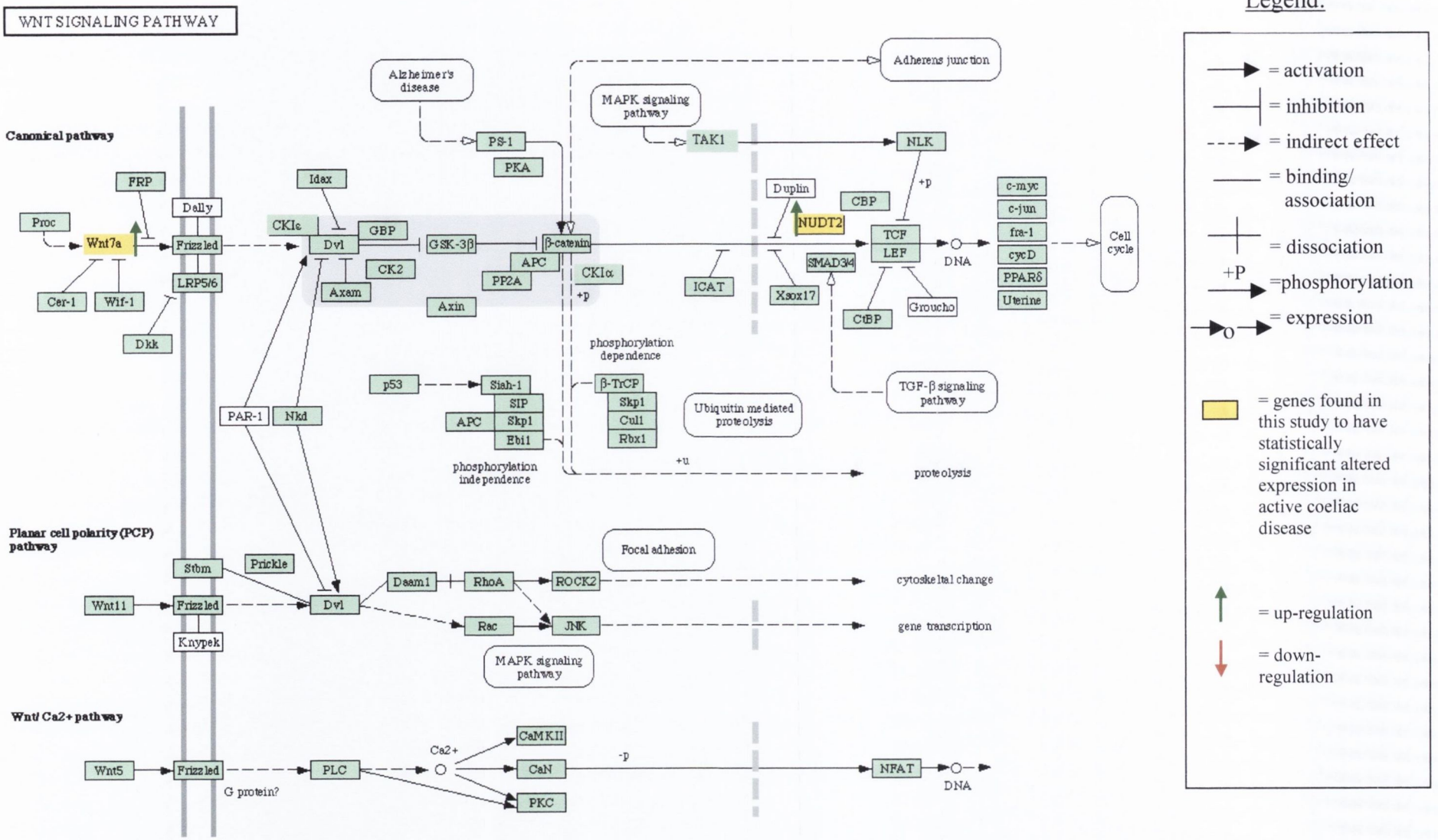


Figure 3.3.10c: Wnt signalling pathway showing involvement of genes with altered expression in active coeliac disease

33 (CELIAC2), Chromosome 2q33 (CELIAC3) and Chromosome 19p13 (CELIAC4) [van Heel *et al.* 2005]. The genes found in these loci and evincing an expression change in the same direction in all experiments were grouped into lists (table 3.3.3a-d).
- Using the gene lists generated from the above three functional analyses, the pathways in which two or more genes participated were examined. The pathway in which the most genes were involved was the apoptosis pathway (Fig. 3.3.10). Many of the pathways found, such as the focal adhesion pathway and Wnt signalling pathway, were interlinked with each other, highlighting the importance of these functional areas (Fig. 3.3.10b & c).

3.3.9.3 Cluster Analysis

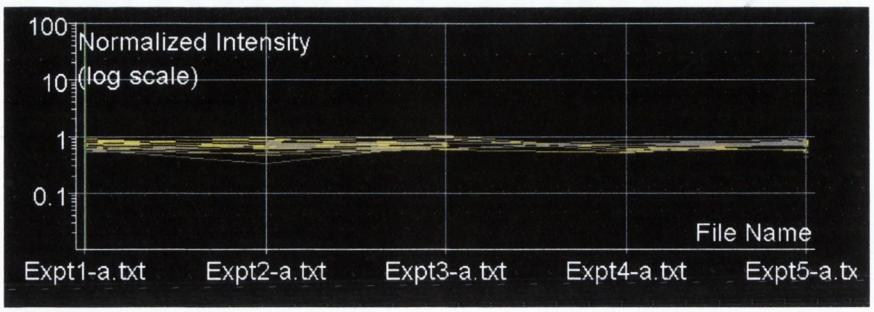
Hierarchical, K-means and QT clustering were performed and assessed for the best quality clusters. With hierarchical clustering, the nodes on the tree can be selected to create a sub-tree or cluster. This selection is manual and therefore not unbiased. Also, there are no quality measures associated with the selected clusters. For these reasons, the hierarchical clustering was deemed unsuitable. The quality of k-means and QT clustering was judged on the basis of how low the radii of the clusters were and how high the percent explained variability was. The scripted best-k-means clustering which produced six clusters, had the highest percent explained variability at 90.822 and the lowest range of radii (table 3.3.4). This set of clusters was used for promoter analysis. Each cluster was analysed for unique transcription factor binding sites (TFBSs) (see Fig 3.3.11).

Table 3.3.4: K-means clusters

Cluster number	Radius of cluster	No. of genes in cluster
1	0.12	25
2	0.121	8
3	0.158	5
4	0.194	9
5	0.189	8
6	0.111	7

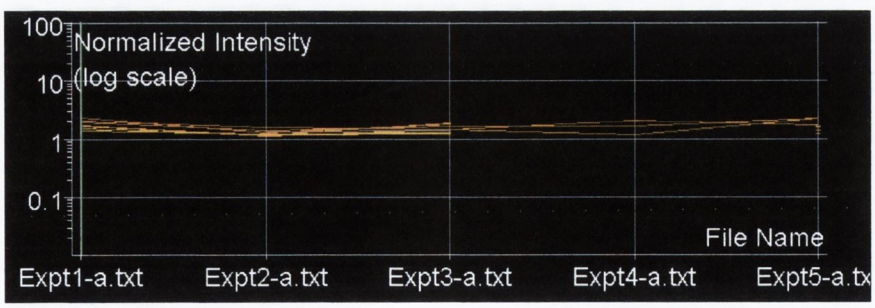
Figure 3.3.11: K-means Clusters showing the genes within and their common transcription factor binding sites (TFBS)

CLUSTER 1



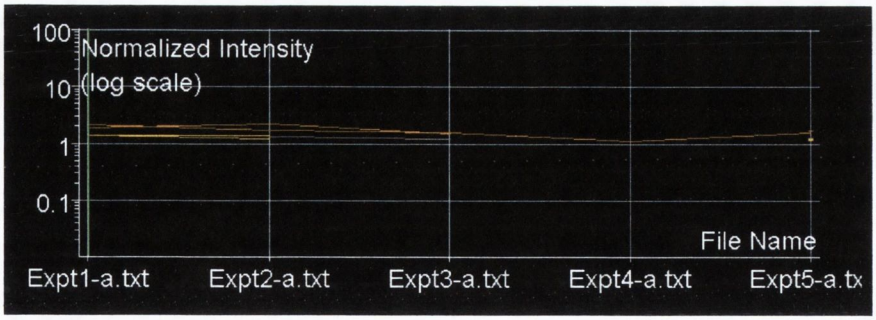
Genes	TFBS
3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 adenosine kinase	BARB
ash2 (absent, small, or homeotic)-like (Drosophila)	
cholinergic receptor, nicotinic, alpha polypeptide 5	
chromosome X open reading frame 2	
dihydropyrimidinase	
envoplakin	
glutamate receptor, ionotropic, N-methyl D-aspartate 1	
glutamate receptor, metabotropic 7	
histatin 1	
MAP/microtubule affinity-regulating kinase 2	
methyl CpG binding protein 2 (Rett syndrome)	
microtubule-associated protein 1A	
N-acetylgalactosaminidase, alpha-	
NADH dehydrogenase (ubiquinone) Fe-S protein 8	
natriuretic peptide receptor A/guanylate cyclase A	
nuclear transcription factor Y, alpha	
prion protein (p27-30) (Creutzfeld-Jakob disease)	
protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4	
PTK2B protein tyrosine kinase 2 beta	
retinol binding protein 1, cellular	
SRY (sex determining region Y)-box 14	
TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor	
tubulin-specific chaperone a	
v-Ha-ras Harvey rat sarcoma viral oncogene homolog	

CLUSTER 2



Genes	TFBS
ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A	P53F
EGF-containing fibulin-like extracellular matrix protein 1	
pyrroline-5-carboxylate reductase 1	
pyruvate kinase, muscle	
ribosomal protein L19	
sialyltransferase 1 (beta-galactoside alpha-2,6-sialyltransferase)	
superkiller viralicidic activity 2-like (S. cerevisiae)	
vinculin	

CLUSTER 3



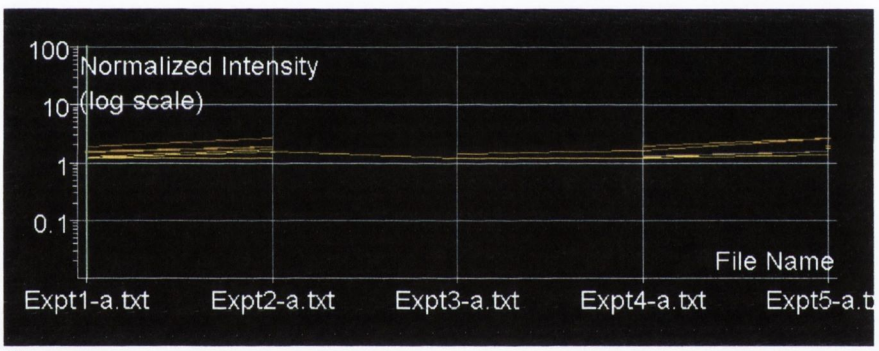
Genes

bone marrow stromal cell antigen 2
microseminoprotein, beta-
Ras-like without CAAX 1
syntaxin 3A
tyrosinase (oculocutaneous albinism IA)

TFBS

AHRR	ATBF
BCL6	EREF
FAST	GREF
HIFF	HOXH
LHXF	LTUP
MYOD	NEUR
OAZF	PERO
PLZF	RARF
SMAD	SRFF
VMYB	

CLUSTER 4



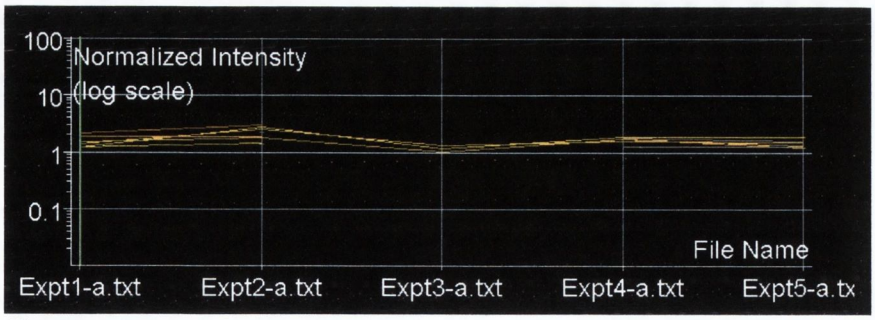
Genes

chromosome 18 open reading frame 1
cytoplasmic linker 2
eukaryotic translation initiation factor 4E binding protein 3
GPI anchored molecule like protein
growth factor receptor-bound protein 7
high-mobility group box 3
integrin beta 4 binding protein
oligodendrocyte myelin glycoprotein
sorcin

TFBS

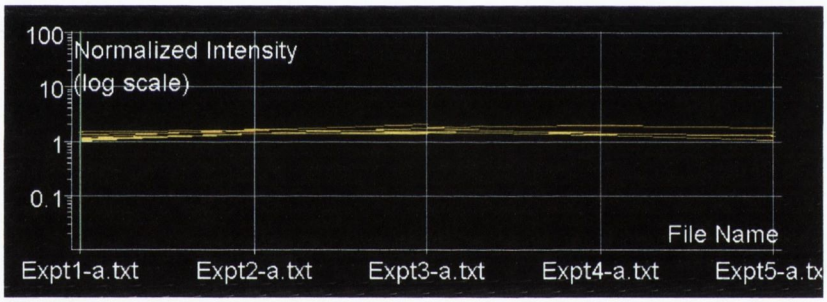
AP1F
LEFF
PBXC
PIT1

CLUSTER 5



Genes	TFBS
ELK4, ETS-domain protein (SRF accessory protein 1)	NF1F
exportin 1 (CRM1 homolog, yeast)	PRDF
G protein-coupled receptor 7	TALE
hematopoietic cell-specific Lyn substrate 1	
homeo box A7	
lipoprotein, Lp(a)	
neuronal PAS domain protein 2	
ribosomal protein S29	

CLUSTER 6



Genes	TFBS
gamma-aminobutyric acid (GABA) A receptor, beta 3	CP2F
histone deacetylase 2	MINI
homeo box A11	MOKF
Notch homolog 3 (Drosophila)	
potassium voltage-gated channel, Isk-related family, member 1	
retinol dehydrogenase 5 (11-cis and 9-cis)	
wingless-type MMTV integration site family, member 7A	

3.4 Discussion

The purpose of this study was to develop a system for hypothesis-free analysis of large numbers of gene transcripts in order to discover novel gene expression and regulation within the enterocyte of active coeliac disease. It is known that the enterocytes are subjected to an inflammatory environment but what we wished to learn more about was the effect this inflammation has on the enterocyte and whether the enterocyte itself contributes to the pathological lesion found in coeliac disease. A system was successfully generated to compare gene expression between two sources and then applied to analyse regulation of gene transcripts in active coeliac disease. A homogeneous population of enterocytes was utilised to ensure we could match the regulated gene transcripts to their cellular source, and thus their part in the inflammatory lesion. A number of genes were found to be significantly up-, or down-regulated in coeliac disease (Table 3.3.1) some of which have previously been reported to be expressed in intestinal epithelial cells such as D-amino-acid oxidase [Thompson *et al.* 1992], delta-aminolevulinate synthase 1 [Fuji *et al.* 2003], choline kinase [Ramírez de Molina *et al.* 2002], hemochromatosis gene [Griffiths and Cox 2003], vinculin [Clark and Hirst 2002], fructose-1, 6-biphosphatase [Yánez *et al.* 2003], and potassium voltage-gated channel, Isk-related family, member 1 [Warth *et al.* 2002].

Functional analysis was carried out on the genes that demonstrated significantly altered expression levels (Fig. 3.3.9). The three main categories that were significantly over-represented amongst the gene list of interest were cell growth and/or maintenance, metabolism and development.

Cell growth and/or maintenance

Seventy-nine percent of the significantly altered genes were shown to be involved in cell growth and/or maintenance, which encompass functions such as proliferation, transport, cell division and cell motility.

It has been suggested that in active coeliac disease, the epithelial crypt cells proliferate at a higher rate than normal [Moss *et al.* 1996]. Growth arrest-specific 6 (GAS6) and bone marrow stromal cell antigen 2 (BST2), which were found in this study to be up-regulated in active coeliac disease, have been previously reported to induce

proliferation of cells via the transcription factor STAT3 [Yanagita *et al.* 2001; Ohtomo *et al.* 1999] and have been demonstrated to be up-regulated in cancer [Wimmel *et al.* 2001; Sainaghi *et al.* 2005; Ohtomo *et al.* 1999; Becker et *al.* 2005]. PRKC, apoptosis, WT1, regulator (PAWR), a gene shown to induce apoptosis of prostate cancer cells, inhibit NF-κB activity [Chakraborty *et al.* 2001] and act as a negative regulator of proliferation [Kuzoc-Zivojnov *et al.* 2004] was found to be down-regulated in active coeliac disease. These results show that the transcriptional regulation of proliferation is altered in coeliac disease.

Alterations in intestinal transport are known to occur in coeliac disease. This study found an increased expression in active coeliac disease, of potassium voltage-gated channel, Isk-related family, member 1 (KCNE1) and calcium channel, voltage-dependent, L type, alpha 1C subunit (CACNA1C), both of which are up-regulated in hypothyroidism [Le Bouter *et al.* 2003] - a condition associated with coeliac disease. Hemochromatosis gene (HFE), the loss of which causes Hereditary Haemochromatosis with iron overload, was found to be up-regulated in active coeliac disease, which would lead to reduced iron uptake, a known symptom of coeliac disease. Decreased expression in active coeliac disease was observed of NADH dehydrogenase (ubiquinone) Fe-S protein 8 (NDUFS8), a gene mutated in Leigh syndrome- an early-onset progressive neurodegenerative disorder [Procaccio and Wallace 2004]. Decreased expression was also found of glutamate receptor, ionotropic, N-methyl D-aspartate 1 (GRIN1), a mutation in which has been linked to schizophrenia [Mohn *et al.* 1999; Qin *et al.* 2005], another condition reported to be associated with coeliac disease [De Santis *et al.* 1997]. N-methyl D-aspartate receptors have been inversely correlated with expression of D-amino-acid oxidase (DAO) [Yoshikawa *et al.* 2004], and an increased expression of DAO in active coeliac disease was demonstrated in this study. Retinol binding protein 1, cellular (RBP1), which transports vitamin A- shown to have a role in epithelial cell differentiation- has been reported to be under-expressed in ovarian and breast carcinomas [Cvetković *et al.* 2003]. Interestingly, this study also showed down-regulation of this gene in active coeliac disease. A number of other genes involved in protein transport were found to be up-regulated in active coeliac disease such as sorcin (SRI), peroxisome biogenesis factor 13 (PEX13), retinol

dehydrogenase 5 (RDH5), gap junction protein, alpha 4, 37kDa (GJA4), syntaxin 3A (STX3A) and exportin 1 (XPO1).

Metabolism

Fifty-five percent of the significantly altered genes have a metabolic function, which includes functions such as primary metabolism (amino acids, carbohydrates, lipids, and protein), cellular metabolism, DNA metabolism and transcription.

A number of genes involved in lipid metabolism were found in this study to be regulated in active coeliac disease. 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (HMGCS1) was down-regulated whereas sterol regulatory element binding transcription factor 1 (SREBP1), lipoprotein, Lp(a) (LPA) and choline kinase (CHK) were up-regulated. Interestingly, choline kinase has been found to be up-regulated in colon, lung, prostate and breast cancer though its exact role in tumorigenesis is not known [Ramírez de Molina *et al.* 2002].

Many transcription factors were found to be up-regulated in active coeliac disease such as Notch homolog 3 (NOTCH3), human T-cell leukemia virus enhancer factor (HTLF), forkhead box G1B (FOXG1B), paired box gene 8 (PAX8), homeobox A11 (HOXA11) and hematopoietic cell-specific Lyn substrate 1 (HCLS1). SRY (sex determining region Y)-box 14 (SOX14) and T-box 6 (TBX6), both involved in regulation of development and embryogenesis [Arsic *et al.* 1998; White and Chapman 2005] were shown here to be repressed in active coeliac disease, while PRKC, apoptosis, WT1, regulator (PAWR) and methyl CpG binding protein 2 (MECP2), both negative regulators of transcription [Johnstone *et al.* 1996; Harikrishnan *et al.* 2005] were also found to be repressed in active coeliac disease. Homeobox A7 (HOXA7), shown to inhibit keratinocyte differentiation [La Celle and Polakowska 2001], was up-regulated in coeliac disease while nuclear transcription factor Y, alpha (NFYA), reported to cause differentiation of CaCo-2 cells through the activation of H-ferritin [Bevilacqua *et al.* 2002], was down-regulated in active coeliac disease. Histone deacetylase 2 (HDAC2), involved in transcriptional repression, has been shown to be over-expressed in colon cancer [Zhu *et al.* 2004] and was found in this study to be up-regulated in active coeliac disease. These results suggest that the many processes

altered during active coeliac disease may be the result of abnormal expression of transcription factors.

Development

Forty-two percent of the significantly altered genes are involved in development which comprises such functions as morphogenesis, cell differentiation, regulation of development and epigenetic regulation of gene expression.

Morphogenesis, the process by which anatomical structures are generated and organised, can occur in embryonic and adult tissue. A number of genes with a function in morphogenesis were found in this study to be up-regulated in coeliac disease. These include alpha-spectrin (SPTA1), homeobox A11 (HOXA11), Notch homolog 3 (NOTCH3), Paired box gene 8 (PAX8) and wingless-type MMTV integration site family, member 7A (WNT7A). WNT7A has been previously shown to be linked to the expression of HOX and NOTCH genes [Calvo *et al.* 2000; Widelitz *et* al. 1999]. WNT7A is involved in several developmental processes and regulates cell fate [Rizvi *et al.* 2005]. On the one hand expression of WNT7A is lost during lung cancer and increased expression is associated with the anti-neoplastic effect of progesterone on endometrial epithelial cells [Oehler *et al.* 2002], and on the other hand WNT7A is over-expressed in head and neck squamous carcinoma [Rhee *et al.* 2002]. Similarly, WNT7A has been reported to stimulate chondrocyte differentiation and inhibit adipocyte differentiation [Zhou *et al.* 2004]. In addition to WNT7A, PAX8 has also been shown to be regulated by TGF-β and is required for the morphogenesis of the thyroid gland [Costamagna *et al.* 2004; Tuli *et al.* 2003].

Cytokines

As shown in chapter 2, pro-inflammatory cytokines are produced in coeliac disease in response to wheat, barley and rye. Many other reports of the involvement of cytokines such as IFN-γ, TNF-α, IL-2, IL-4, IL-6 and IL-10 in coeliac disease exist [Kontakou *et al.* 1995; Nilsen *et al.* 1995; Nilsen *et al.* 1998; Troncone *et al.* 1998; Westerholm-Ormio *et al.* 2002; Forsberg *et al.* 2002]. Most studies have concluded that the source of the pro-inflammatory cytokines is the Th1 cells in the lamina propria. Despite the

fact that intestinal epithelial cells have been shown to produce a number of cytokines (see section 1.1.2), the only cytokine reported to be up-regulated in coeliac intestinal epithelial cells is IL-15 [Maiuri *et al.* 2000]. For this reason expression of genes encoding cytokines, cytokine receptors and proteins induced by cytokines was examined. Genes evincing an expression change in the same direction in all experiments but which did not reach the statistical significance cut-off were also inspected (Table 3.3.2), although it should be noted that the more statistically significant a result is, the more likely it is to represent a true outcome.

Interleukins 3, 17 and 18 were up-regulated in coeliac disease, of which IL-3 and IL-18 have previously been reported to be expressed in epithelial cells [Dalloul *et al.* 1991; Kalina *et al.* 2002]. Furthermore, IL-18, produced by intestinal epithelial cells has been shown to cause proliferation of intra-epithelial T-cells [Okazawa *et al.* 2004]. Additionally, IL-18, capable of promoting T-cell IFN-γ production and facilitating Th1 cell polarisation, was previously shown to be up-regulated in coeliac biopsies, although the cell of origin was not determined [Salvati *et al.* 2002]. Members of the IFN-α family, 8 and 16, were found to be up-regulated in active coeliac disease. Treatment of chronic hepatitis C with IFN-α has led to development of coeliac disease in some patients [Cammarota *et al.* 2000] and in fetal gut explants, IFN-α caused crypt cell hyperplasia and enhanced Th1 responses [Monteleone *et al.* 2001a].

These results show that epithelial-derived cytokines may have a greater role in the pathogenesis of coeliac disease than expected. IL-12, considered the major cytokine in promoting the development of Th1 cells is undetected in coeliac gut mucosa [Monteleone 2001b], thus IL-18 or IFN-α produced by epithelial cells may be a promoter of Th1 responses in coeliac disease.

A number of interleukin receptors were found to be up-regulated in active coeliac disease, as well as the interleukin-1 receptor-associated kinases (IRAKs) 1 and 2. As described in the apoptosis pathway, these kinases become associated with the IL-1 receptor upon stimulation and are involved in the up-regulation of NF-κB. IRAK-1 has also been shown to be involved in the activation of macrophages [Cuschierei *et al.* 2004]. IL-6 signal transducer (also known as gp130) which forms part of the IL-6

receptor complex was up-regulated in active coeliac disease. This gene is expressed in breast cancer cells and activates the transcriptional factor STAT3 which regulates numerous important cellular genes involved in growth regulation, differentiation, and cell cycle, including c-myc and bcl-2 [Selander *et al.* 2004].

Two members of the TNF receptor super-family were up-regulated in active coeliac disease: TNFRSF18, which activates NF-κB and TNFRSF11B (also known as osteoprotogerin), which functions as a negative regulator of bone resorption and an apoptotic factor, and has been found over-expressed in intestinal epithelial cells [Ariyasu *et al.* 2002] and prostate cancer cells [Holen *et al.* 2002]. A number of TNF super-family ligands such as TNFSF12 and 13 were also up-regulated in active coeliac disease.

A group of genes directly induced by cytokines were found to be regulated in active coeliac disease. Interferon alpha-inducible protein 27 (IFI27), a gene which localises to the nuclear envelope upon treatment with IFN-α, is up-regulated in coeliac disease. This gene is described as a marker of epithelial proliferation and was shown to be up-regulated in psoriasis and epithelial skin cancers [Suomela *et al.* 2004]. Other interferon-inducible genes found to be up-regulated in active coeliac disease were guanylate binding protein 1, interferon-inducible (GBP1) and myxovirus resistance 1, interferon-inducible protein p78 (MX1) shown to mediate anti-viral effects against vesicular stomatitis virus and encephalomyocarditis virus [Anderson *et al.* 1999] and influenza virus [Turan *et al.* 2004], respectively. Tumour necrosis factor, alpha-induced protein 3 (TNFAIP3), an inhibitor of apoptosis and previously reported as expressed in follicle-associated epithelium [Anderle *et al,* 2005], was also found to be up-regulated in active coeliac disease.

Chromosomal analysis

Genome wide linkage studies have been performed in coeliac disease in an attempt to identify chromosomal regions likely to contain disease causing genes. The HLA region (6p21) has been repeatedly reported to have strong linkage while convincing evidence for linkage in the non-HLA regions 5q31-33, 2q33 and 19p13 exists [van Heel *et al.*

2005]. Candidate gene association studies within these loci have concentrated on genes known to be immunologically relevant to disease pathogenesis, yet so far no gene has been conclusively proven to confer a risk of coeliac disease. A more hypothesis-free approach to selecting genes for study may be useful.

6p21 (CELIAC1):

A number of studies have examined whether there are additional alleles in this locus that may confer increased disease risk over and above HLA-DQ. Numerous potential candidate genes with an immune function exist in this region such as tumour necrosis factor (TNF) α and β, and MHC class I polypeptide-related sequence (MIC) A and B; however, reports conflict as to whether any disease risk is conferred by these genes [McManus *et al.* 1996; Fernandez *et al.* 2002; Louka *et al.* 2003]. None of the previously studied genes within this region were found to be differentially expressed in this study. Twenty-seven genes evincing an expression change in the same direction in all experiments were found to have altered expression in active coeliac disease (table 3.3.3a), of which three were significantly altered. These were hemochromatosis (HFE), nuclear transcription factor Y, alpha (NFYA) and superkiller viralicidic activity 2-like (SKIV2L). The functions of the first two genes are described above under cell growth and/or maintenance and metabolism respectively, while SKIV2L is reported to confer resistance to killer toxin RNA viruses [Gruen and Weissman 2001]. Other interesting differentially expressed genes which do not reach statistical significance are: nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1 (NFKBIL1 aka IKBL), a gene likely to have a function similar to the NF-κB inhibitor, I-κBα and in which polymorphisms have been demonstrated in Multiple Sclerosis and Rheumatoid Arthritis [Miterski *et al.* 2002; Okamoto *et al.* 2003]; proteasome subunit, beta type, 8 (PSMB8 aka LMP7), an IFN-γ-dependent gene which plays an essential role in the processing of major histocompatibility complex class (MHC) I ligands and has been shown to be associated with Type 1 Diabetes Mellitus and the autoimmune skin disease, Vitiligo [Ding *et al.* 2001; Casp *et al.* 2003]; and BCL2-antagonist/killer 1 (BAK1), a pro-apoptotic gene found to be down-regulated in this study.

5q31-33 (CELIAC2):

This is the region which has been linked most consistently to coeliac disease with reproducible evidence coming from extensive family studies [Ryan *et al.* 2005]. Some of the genes analysed for an association in coeliac disease are IL4, IL5, IL9, IL12B, IL13, IL17B, NR3C1 and SLC22A; however, no evidence for an association was found [Louka *et al.* 2002; Ryan *et al.* 2004; Ryan *et al.* 2005]. None of these genes were found to be differentially expressed in this study, although it should be noted that these interleukin genes are not known to be expressed by epithelial cells. Eleven genes were, however, found to be differentially expressed and evincing an expression change in the same direction in all experiments (table 3.3.3b), none of which had significantly altered expression. Some interesting genes were differentially expressed but did not reach statistical significance, for example: IL-3, a cytokine which regulates the growth, differentiation and activation of hematopoietic progenitor cells and mature macrophages, and in which a single-nucleotide-polymorphism has been demonstrated in Rheumatoid Arthritis [Yamada *et al.* 2001]; glycine receptor, alpha 1 (down-regulated in coeliac disease), a gene involved in chloride transport and shown to be mutated in the neurological condition, Hereditary hyperekplexia [Gilbert *et al.* 2004]; and IL2-inducible T-cell kinase (ITK), involved in mast cell degranulation and acute allergic responses [Forssell *et al.* 2005].

2q33 (CELIAC 3):

This genomic region containing the CTLA-4, ICOS and CD28 genes has shown linkage to coeliac disease in several genome wide scans and replication studies [van Heel *et al.* 2005]. The candidate gene CTLA-4 has received the most attention; however, of the 14 association studies performed on this gene; only four have demonstrated an association [van Heel *et al.* 2005]. Ten genes were found in this study to be differentially expressed and evincing an expression change in the same direction in all experiments (table 3.3.3c), none of which had significantly altered expression. The previously studied genes in this region did not occur amongst these ten genes. Some interesting genes which were differentially expressed but did not reach statistical significance are: caspase 8 (found to be down-regulated in active coeliac disease), a pro-apoptotic gene in which mutations have been found in colorectal cancer [Kim *et al.* 2003]; and the heat-shock protein genes heat shock 10kDa protein 1 (HSPE1) and

DnaJ (Hsp40) homolog, subfamily B, member 2 (HSPF3), both involved in chaperone functions while the former has a function in protecting myocytes from simulated-ischemia [Lin *et al.* 2004].

19p13 (CELIAC4):

One of the largest and most recent studies, performed in a Dutch cohort of coeliac patients meeting strict diagnostic criteria, identified significant linkage to the region 19p13 [van Belzen *et al.* 2004]. As yet no genes in this region have been reported to have been analysed for association. Nine genes were found in this study to be differentially expressed and evincing an expression change in the same direction in all experiments (table 3.3.3d), none of which had statistically significant altered expression. Two genes encoding zinc finger proteins, involved in gene regulation and development were differentially expressed, ZNF43 (down-regulated in active coeliac disease and ZNF91 (up-regulated in active coeliac disease) – also shown to have a proliferative and anti-apoptotic role in acute myelogenous leukemia [Unoki *et al.* 2003].

Pathways

Pathways detailing the sequence of events in biological processes were examined for involvement of any of the altered genes listed in tables 3.3.1, 3.3.2, 3.3.3a-d and discussed above.

The biological pathway in which the highest number of altered genes participates is the **apoptosis pathway** (Fig. 3.3.10a). Apoptosis can occur through the intrinsic pathway, initiated through stress signals which cause mitochondrial release of apoptotic factors; or the extrinsic pathway, initiated through pro-inflammatory cytokines and death ligands which activate the caspase cascade. Caspase 8, a central molecule in this pro-apoptotic pathway was found in this study to be down-regulated in active coeliac disease. The pro-inflammatory cytokines and death ligands can also activate an opposing pathway which causes the activation of NF-κB and results in cell survival. Interleukin-1 receptor associated kinase (IRAK), found here to be up-regulated in active coeliac disease, participates in this anti-apoptotic pathway and leads to the phosphorylation of IκBα which then releases and activates NF-κB.

A number of growth factors can promote cell survival through the inhibition of the pro-apoptotic protein, Bad, and through the activation of NF-κB. In this study, the survival factors erythropoietin (EPO) and interleukin 3 (IL-3) were found to be up-regulated in active coeliac disease. Hypoxia-stimulated expression of EPO has been reported in human breast and cervix carcinomas, suggesting a role for EPO signalling in the survival of cancer cells [Acs *et al.* 2004].

Protein tyrosine kinase 2 beta (PTK2B), when activated by a rise in intracellular calcium or stress signals, leads to apoptosis. Inhibition of PTK2B has been found to block TNF-α induced apoptosis in neutrophils [Avdi *et al.* 2001]. PTK2B was found here to be down-regulated in active coeliac disease, as was the pro-apoptotic gene BCL2-antagonist/killer 1 (BAK1). BAK1 has previously been shown to be down-regulated by viral oncoproteins in laryngeal carcinoma associated with human papilloma virus (HPV) [Du *et al.* 2004], suggesting that the dysregulation of apoptotic molecules contributes to the prolongation of cell survival.

A number of genes with altered expression in active coeliac disease participate in the **focal adhesion pathway** (Fig.3.3.10b), which interacts with many biological pathways including apoptosis and Wnt signalling. Focal adhesions are found at the cell membrane where the cytoskeleton interacts with proteins of the extracellular matrix. The components of focal adhesions are diverse and include scaffolding molecules, GTPases, and enzymes such as kinases, phosphatases, proteases, and lipases. Initiated in cells by interaction with the extracellular matrix or pro-inflammatory cytokines, the complex focal adhesion pathway can regulate cell migration, proliferation and survival. Formation of focal adhesions is necessary for anchor-dependent cell survival, and both assembly and disassembly of focal adhesions is required for cell migration. In normal cells these processes are regulated to occur at the necessary times but in transformed, cancerous cells the processes become dysregulated where increased migration occurs and cells become capable of anchorage-independent survival, leading to metastasis.

PTK2B, also known as focal adhesion kinase (FAK) 2, can participate in a number of key positions controlling the end-processes of the pathway. This enzyme causes cell migration via reorganisation of focal adhesions and cell rounding [Du *et al.* 2001] while its inhibition leads to decreased cell spreading and reduced migration [Di Cioccio 2004; Owen *et al.* 1999]. In the study presented here, PTK2B was found to be down-regulated in active coeliac disease. Proliferation is indirectly regulated by PTK2B and may be increased or decreased depending on the cell and location. For example, in prostate carcinoma, PTK2B expression inversely correlates with the degree of malignancy with PTK2B expression progressively decreasing in increasingly high grade adenocarcinoma until disappearing in anaplastic undifferentiated cancer [Stanzione *et al.* 2001]. In contrast, increased PTK2B expression in astrocytomas correlates with an increase in grade of malignancy [Gutenberg *et al.* 2004].

Vinculin (VCL), an actin-binding cytoskeletal protein involved in formation of focal adhesions and cell-cell adhesion, was found to be up-regulated in active coeliac disease. Up-regulation of vinculin has been shown to inhibit cell motility [Rodriguez-Fernandez *et al.* 1992]. Choline kinase, also found to be up-regulated in active coeliac disease, is activated by RhoA through ROCK kinase and leads to the assembly of focal

adhesions. Over-expression of choline kinase has been demonstrated to induce both anchorage independent growth and tumorigenicity with an oncogenic potential similar to that of RhoA alone [Ramírez de Molina *et al.* 2005].
Finally, the pro-apoptotic molecule BAK1, which is inhibited by MEK1 and Raf-1 in the focal adhesion pathway, was found to be down-regulated in this study in active coeliac disease. The above findings in relation to the focal adhesion pathway highlight key processes that may increase cell adhesion and survival in the coeliac epithelium.

Another pathway involving a number of genes significantly altered in active coeliac disease is the wingless-type MMTV integration site or **Wnt signalling pathway** (Fig.3.3.10c). This pathway interacts with the focal adhesion pathway and incorporates the significant functional area of development (discussed above). The Wnt pathway along with Notch, TGF-β and Hedgehog pathways plays a key role in the homeostatic control of the adult intestinal epithelium. Many hereditary cancers disrupt these signalling cascades through mutational activation and mutations in these pathways are also found in sporadic tumours [Taipale and Beachy 2001].

In the canonical Wnt pathway, Wnt7a - found in this study to be up-regulated in active coeliac disease- is released from or presented on the surface of signalling cells and binds to the Frizzled/low density lipoprotein receptor-related protein complex at the cell surface of target cells. These receptors then appear to release a signal that inhibits a number of proteins residing in the APC complex, such as Dishevelled (DVL), glycogen synthase kinase-3β (GSK-3β), Axin and Adenomatous Polyposis Coli (APC) which prevents them from sequentially phosphorylating β-catenin and causing it to become a target for rapid proteasome-mediated degradation. Thus Wnt signalling leads to an accumulation of β-catenin in the cytoplasm which then translocates to the nucleus where it interacts with transcription factors such as members of the T-cell factor/lymphoid enhancing factor (TCF/LEF) family. The downstream effects of activation of these transcription factors are diverse and are often determined by the cell type and location. Wnt activation of TCF-4 has been shown to cause proliferation of intestinal epithelial cells, with nuclear localisation of β-catenin observed throughout the bottom third of the crypts including base columnar, paneth and putative stem cells

[Hauck *et al.* 2005]. Mutational activation of the Wnt signalling pathway in the intestine occurs in Familial adenomatous polyposis, an autosomal dominant disease in which patients display hundreds or thousands of polyps in the colon due to increased cell proliferation.

Wnt7a has been reported to be up-regulated in wounded cornea where it leads to the proliferation of corneal epithelial cells and enhanced wound closure [Lyu and Joo 2005]. Wnt7a signalling was shown to induce the transcription of metalloelastase (MMP-12) which when blocked delayed the wound closure. MMP-12 has previously been reported to be expressed by sub-epithelial macrophages in the intestine of coeliac patients and in macrophages migrating towards and into the epidermis of blisters in patients with dermatitis herpetiformis [Salmela *et al.* 2001]. Interestingly, MMP-12 is capable of activating latent TNF-α, which is known to have a role in the pathogenic lesion in coeliac disease [Chandler *et al.* 1996].

Nudix (nucleoside diphosphate linked moiety X)-type motif 2 (NUDT2) which cooperates with β-catenin in the transcription of TCF-regulated genes was found to be up-regulated in active coeliac disease. NUDT2 is thought to have a role in cell survival, which is likely to occur through TCF1-regulated Bcl-2 expression.

Transcription factor binding sites (TFBSs)

In eukaryotes, regulation of gene expression is achieved through the complex interaction of regulatory proteins (transcription-factors) with specific DNA binding sites (*cis*-acting regulatory sequences). Clustering of microarray data can be used to identify *cis*-regulatory elements. The rationale is that co-expressed genes are likely to be co-regulated and, therefore, may share common regulatory elements. In this study, k-means clustering retrieved six clusters of genes with different expression profiles (Fig.3.3.11). The transcription factor binding sites (TFBSs) common to each gene in the cluster and unique to a single cluster were determined. In clusters with larger lists of genes such as cluster 1, the likelihood of finding TFBSs common to all genes is reduced thus larger gene lists led to fewer common TFBSs and vice versa.

Cluster 1 contains 25 genes down-regulated in active coeliac disease. The single TFBS found in common amongst these genes was BARB (barbiturate-inducible element, aka barbie-box). All genes that encode barbiturate-inducible proteins contain the Barbie-box element [Liang *et al.* 1995], thus these 25 genes are induced by barbiturates. One such barbiturate, Phenobarbital, is known to induce cytochrome P450, a family of liver enzymes with an important role in metabolic detoxification and elimination [Waxman and Azaroff 1992; Liang *et al.* 1995]. Pro-inflammatory cytokines such as IFN-γ, TNF-α and IL-6 have been shown to reduce P450 expression, thought to occur by decreasing the level of phenolbarbital-induced transcription factor CAR (constitutively activated receptor) [van Ess *et al.* 2001]. It is therefore possible that the pro-inflammatory cytokines found up-regulated in active coeliac disease are causing down-regulation of the genes found in cluster 1.

Cluster 2 contains eight genes up-regulated in coeliac disease. P53F was the only common TFBS found in these genes. p53 is a transcription factor traditionally known as a tumour suppressor gene which induces cell-cycle arrest and apoptosis in response to DNA damage [Yonish-Rouach *et al.* 1991; Steele and Lane 2005]. It has also been reported that p53 is not only involved in the elimination of terminally damaged cells but may also contribute to the proliferative response that allows regeneration of the damaged tissue [Shin *et al.* 1995; Inoue *et al.* 2002; Panasiuk *et al.* 2005]. This regeneration has been shown to occur through the secretion of p53-responsive growth

factors such as transforming growth factor (TGF)-α, epidermal growth factor (EGF) receptor, and hepatocyte growth factor (HGF) [Shin *et al.* 1995; Inoue *et al.* 2002; Panasiuk *et al.* 2005]. The genes in cluster 2 have functions in cell growth and/or maintenance; thus, it is likely they participate in p53-induced regeneration of the epithelium.

Cluster 3 consists of five genes up-regulated in active coeliac disease. Nineteen TFBSs were found in common in these genes. Many of these transcription factors have a function in cell growth, proliferation and differentiation. FAST (fork head activin signal transducer) and SMAD (SMAD, mothers against DPP homolog) complex with one another and transduce TGF-β signalling, known to have a role in growth inhibitory and differentiation processes [Liu *et al.* 1999]. HIF (hypoxia-inducible factor), activated during hypoxic conditions as well as by cytokines and growth factors, transcriptionally up-regulates genes involved in glycolytic energy metabolism, cell growth and survival, angiogenesis and erythropoiesis [Dalgard et al. 2004]. PERO (peroxisome proliferator-activated receptor aka PPAR) is a transcription factor involved in the regulation of multiple processes including differentiation, cholesterol transport, tumorigenesis, wound-healing and inflammation [Soon Tan et al. 2005].
Cluster 4 encompasses nine genes up-regulated in active coeliac disease, with four TFBSs in common. AP1F (activator protein 1), PBXC (pre-B-cell leukaemia transcription factor) and LEFF (lymphoid enhancer factor) are involved in regulating growth and differentiation processes. The activator protein 1 (AP-1) transcription factor is mainly composed of Jun, Fos and ATF protein dimers, and mediates gene regulation in response to physiological and pathological stimuli, including cytokines, growth factors, stress signals, bacterial and viral infections, as well as oncogenic stimuli [Hess *et al.* 2004]. Pre-B cell leukemia transcription factors (PBXs) act as cofactors in the transcriptional regulation mediated by Homeobox proteins during embryonic development and cellular differentiation [Qin *et al.* 2004]. Lymphoid enhancer factors (LEFs) act as transcriptional regulators in the Wnt signalling pathway (see above) and are involved in cell proliferation and survival [Reya *et al.* 2000].

Cluster 5 contains eight genes up-regulated in active coeliac disease which have three TFBSs in common, PRDF (positive regulatory domain binding factor), NF1F (nuclear

factor 1) and TALE (three-amino acid loop extension). NFI proteins have been implicated in the control of cell growth and over-expression of NFI has been shown to decrease the inhibitory function of TGF-β [Gronostajski 2000; Baldwin 2004]. TALE homeodomain proteins are a class of transcription factors that participate in multiprotein complexes to regulate developmental gene expression. TG-interacting factor (TGIF), a member of this class functions as a transcriptional co-repressor that associates with TGF-β-activated Smad proteins Smad2 and Smad3 and represses TGF-β-activated transcription through these Smad proteins [Chen et al. 2003].

Cluster 6 consists of seven genes up-regulated in active coeliac disease. Three TFBSs were found in common in these genes. MINI (muscle initiator sequence) has not yet had binding proteins identified [Frith et al. 2004]. MOKF (Mouse Kruppel like factor) is a zinc finger protein which can recognise both DNA and RNA [Arranz *et al.* 1997]. CP2F (transcription factor CP2 aka LSF) is involved in development as well as cell growth and survival and induces the expression of thymidylate synthase, essential for DNA replication [Kang *et al.* 2004; Powell *et al.* 2000].

Methodology

The microarrays used in this study were Atlas Glass Human 3.8 I microarrays (BD Biosciences Clontech, UK). A number of factors affected the choice of microarray system used; firstly, at the time of initiation of this study, far fewer commercial companies were producing microarrays. Secondly, the limiting starting material (<2.5μg total RNA per patient) meant that a microarray system which incorporated an amplification step in the preparation of labelled sample nucleic acid was required. A disadvantage of using this system is that the inclusion of an amplification step may introduce mistakes that could distort the data; however, the SMART PCR-based exponential amplification used in this system (section 3.2.8) has been shown to be the most reliable RNA amplification technique [Subkhankulova and Livesey 2006]. The third factor affecting the choice of microarray system was the method of detection. Fluorescent detection was chosen as it is safe, simple and extremely sensitive with high resolution. A disadvantage of fluorescence can be a reduction in quantitative accuracy of the assay, where the incorporation of the label may alter binding properties. This phenomenon was avoided in this study by performing a dye-swap replicate experiment with each sample pair; thus, any discrepancies in rates of incorporation of the different dyes during the labelling step are controlled for (3.2.11).

The method used in this study to distinguish significantly expressed genes was to generate statistical p-values for each gene and place a cut-off of significance at a p-value of 0.05 or less (95% confidence). P-values are the most commonly reported statistical measure and describe the probability that a difference between two groups during an experiment occurred by chance only. In multiple testing experiments such as a microarray where many genes are tested simultaneously, there is a large multiplicity issue and an increased chance of errors occuring. In any testing situation, two types of error can be encountered: a false positive, or Type I error, is committed by declaring a gene to be differentially expressed when it is not, and a false negative, or Type II error, is committed when a truly differentially expressed gene is failed to be identified. In multiple testing situations, the chance of committing a type I error increases with the number of tests performed.

A number of multiple testing corrections have been generated which control the type I error rate at different levels. The Family-wise error-rate (FWER) is defined as the overall probablilty that at least one gene is incorrectly identified as differentially expressed over a number of tests. The single-step Bonferroni correction is the best known correction to control FWER and defines an effective rate as the standard false positive rate divided by the number of tests conducted, which in this study is 0.05/1256 = 0.0000398. Thus to ensure the probablilty of making at least one false positive error among the set of statistical tests is no more than 0.05, only p-values of less than or equal to 0.0000398 are accepted. This correction is an extremely stringent control that drastically increases the false negative rate and therefore may not be acceptable for microarray data.

The False-discovery rate (FDR) is defined as the probablilty that a given gene identified as being differentially expressed is a false positive. Benjamini and Hochberg [1995] first proposed a step-down procedure to control the FDR. Using this method the FDR is calculated as the number of false positive tests at a specific p-value divided by the number of tests called significantly expressed at that p-value. In this study, the FDR is (0.05 x 1256)/ 102 = 0.616. However, this is now considered a conservative estimate that assumes all null hypotheses are true, that is, none of the genes are differentially expressed. Storey [2002] argued that it is more appropriate to consider the positive FDR (pFDR), which incorporates an estimated proportion of truly null hypotheses by using information from the data. This multiple testing correction, considered to give greater power than Benjamini and Hochberg's correction [Koen *et al.* 2005], adjusts the FDR using Π_0, the overall proportion of true null hypotheses in the study. This proportion can be estimated in the QVALUE software written by Storey [Storey *et al.* 2003], and for this study was calculated to be 0.3283. Thus, the pFDR for this study is 0.3283 x (0.05 x 1256)/ 102 = 0.202. This rate therefore indicates that 20.2% of the genes described as statistically significant are false positives. Interestingly, when validation experiments were performed using RT-PCR (chapter 4), 80% of the genes were confirmed to be differentially expressed, thus corroborating the estimated error rate.

The issue as to which multiple testing correction to use, if any, still remains. DNA microarray experiments are generally used as a first exploratory step in the process of identifying subsets of genes involved in important biological processes. In these circumstances, it may be more relevant to minimise the type II errors rather than minimise the type I error, that is, a larger number of false positives may be tolerated in order to find as many interesting genes as possible. Future multiple testing situations will likely be optimised to minimise the type II error rate at a given level of type I error [Dudoit *et al.* 2003].

DNA microarray technology has the potential to aid research in many areas such as gene discovery, gene regulation, disease diagnosis and classification, and drug discovery and toxicity. The high through-put nature of microarrays allows for faster and more extensive analysis as well as avoiding the hypothesis-driven nature of selecting individual candidate genes. Apart from inherent technical difficulties and prohibitive costs of performing microarray studies, there are a number of limitations to using this technology. Firstly, DNA microarrays measure changes in the levels of mRNA expression which does not necessarily represent the level of protein production. Post-transcriptional and post-translational modification can occur; thus, mRNA analysis may not correlate to the regulation of protein activity. To definitively conclude a differentially expressed gene has a biological function, protein expression must also be analysed. Secondly, the differences in methodology between studies make comparing results difficult. Experimental designs, types of microarray and analysis tools vary greatly and can lead to different genes being reported as differentially expressed. Currently no consensus standards exist. The process of filtering "meaningful" data is perhaps the area with the least agreement. Most investigators filter data using some or a combination of the following: signal confidence (an index of signal quality), fold change, minimum acceptable signal intensity, and a statistical cut-off [Cook and Rosenzweig 2002]. While the need to restrict the data to a select list of genes is obvious for display purposes in publications as well as for reducing the computational workload in functional analysis, it may result in potentially important genes being ignored. Most microarray studies are performed as hypothesis-generating experiments; thus, the need for a stringent cut-off point to

identify relevant genes is less important than the need to perform perform validation and follow-up studies to confirm the biological significance of a gene.

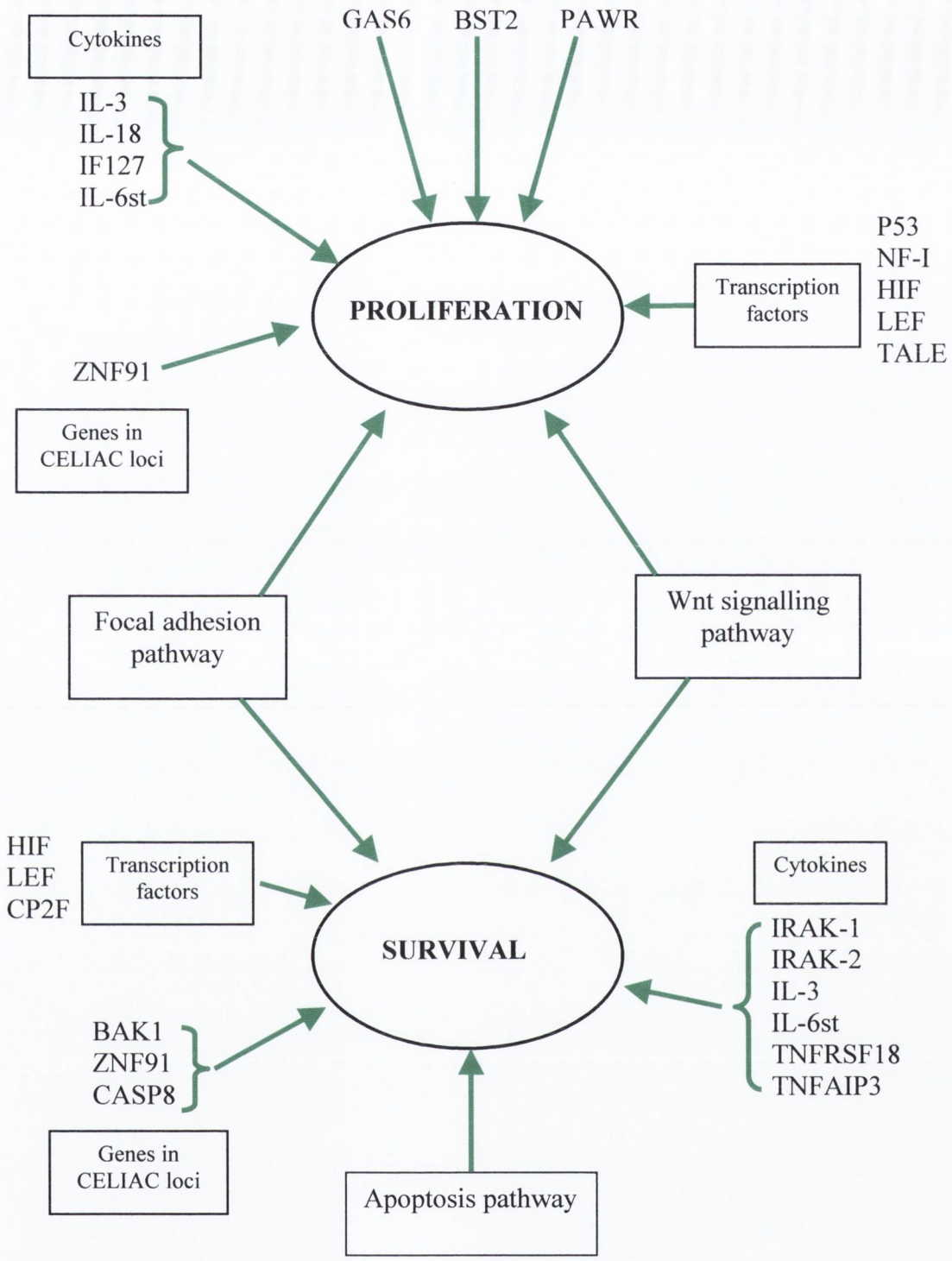


Figure 3.4.1: Overview of key processes found to be regulated at the molecular level.

Summary and Conclusions

The main functional categories found in the significantly altered genes were cell growth and/or maintenance, metabolism and development. The biological pathway in which the most altered genes participated was the apoptosis pathway. Other biological pathways found to interact with this pathway and which had a number of altered genes participating were focal adhesion and Wnt signalling. From these functional analyses a number of processes appear to be repeatedly regulated. Firstly, proliferation would seem to be increased while differentiation decreased. This is in keeping with findings of other microarray studies in coeliac disease [Juuti-Uusitalo *et al.* 2004; Diosdado *et al.* 2004], although these studies used whole biopsies as starting material; thus, it cannot be concluded which cell was expressing the genes. Secondly, survival appeared to be increased while apoptosis was decreased. Examination of genes with a cytokine function and genes found in the CELIAC loci, although often not reaching statistical significance, also showed an up-regulation of proliferation and survival. Transcription factor binding site analysis also showed that many transcription factors regulating these processes are active in coeliac disease (Fig. 3.4.1)

In the normal small intestine, cell proliferation, differentiation and death occur in an orderly fashion along the crypt-villous axis. In the crypt compartment, stem cells are continuously proliferating to provide enough cells for the epithelium to renew itself every five days [Rizvi *et al.* 2005], and survival of these crypt cells is key for the epithelium to maintain its self-renewing capacity. Crypt cells are undifferentiated and only become mature, differentiated cells when they migrate up into the villous compartment. Therefore the gene expression profile found in this study appears to be more descriptive of crypt cells than villous cells. This may be because larger crypts and fewer or absent villi exist in the small intestine of active coeliac disease (section 1.2.2). Crypt hyperplasia is an important feature of the coeliac intestinal lesion and is considered to be the first architectural change in the pathogenic process [Oberhuber *et al.* 1999]. The adaptive immune response is known to play a role in the pathogenesis of the coeliac lesion, and villous atrophy has previously been attributed to being a consequence of the release of epithelial cell-damaging pro-inflammatory cytokines by activated T-cells in the lamina propria. However, this does not explain the initiation of crypt hyperplasia. It is possible that in coeliac disease, the regulation of processes in

the crypt becomes skewed leading to enlarged, malfunctional crypts. Villous atrophy could then be a consequence of a failure to provide mature epithelial cells capable of surviving in the harsh environment of the intestine in which the villus protrudes.

In the normal villus, a certain amount of apoptosis occurs naturally when terminally differentiated cells are extruded into the intestinal lumen from the villous tip. However, a number of studies have reported an increase in apoptosis in the coeliac epithelium [Moss *et al.* 1996; Ciccocioppo *et al.* 2001; Maiuri *et al. 2003*]. This is in contrast to the findings of the study presented here and may be explained by the different features of crypt and villous-tip epithelial cells. Villous-tip epithelial cells are likely to be exposed to more stress signals, particularly gliadin- which has been reported to cause oxidative stress in epithelial cells [Rivabene *et al.* 1999]- and thus may become susceptible to apoptosis through the intrinsic pathway. Crypt epithelial cells which are in a proliferative state may block apoptosis and increase survival.

A number of anti-apoptotic or survival mechanisms were found to be up-regulated in this study. It is likely that genes involved in survival are expressed to a certain degree in the normal small intestinal crypt. However, the up-regulation of survival factors and concurrent down-regulation of apoptotic factors in coeliac epithelium is beyond the normal requirement of crypt cells to survive and could result in preventing damaged cells from undergoing apoptosis. There is an increased rate of apoptosis in the normal small intestinal epithelium compared to the large intestine, where apoptosis is thought to be blocked by Bcl-2 [Booth and Potten 2000]. This is most likely the reason that a far greater number of cancers occur in the large intestine. Many genes discussed in this study are regulated in a similar manner as in cancerous cells, where increased proliferation and decreased apoptosis occurs. Hyperplastic crypts are also found in the colon and are now considered to be pre-cancerous [Nascimbeni 1999; Jass 2000]. With a similar profile of processes as in cancer, it would seem likely that cancerous growths could occur in the coeliac small intestinal epithelium. An increased incidence of cancers such as adenocarcinoma (between 10-fold and 80-fold) is reported in coeliac disease [Swinson *et al.* 1983; Askling *et al.* 2002; Rampertab *et al.* 2003]. However, the average annual age-adjusted incidence of small intestinal adenocarcinoma is estimated to be between 3.7 and 14 per million persons [Chow et al. 1996; Hutchins et

al. 2001]; thus, only a minority of coeliac patients will be affected by these types of cancers.

A number of genes involved in transport and metabolism were also found to be differentially expressed. As the epithelial layer in active coeliac disease is in disarray, in particular the villous compartment, it is to be expected that the normal functions of these cells would become disordered. Alterations in metabolism may reflect the modified needs of the rapidly renewing cells. Whether alterations in transport are a symptom of the disease or a cause is unknown. Ion transport is known to be increased in coeliac disease [Schulzke *et al.* 1995] and an up-regulation of genes involved in ion channels was found in this study. Villous epithelial cells mediate the processes of net nutrient, electrolyte and fluid absorption while crypt cells actively secrete fluid and electrolytes [Sandle *et al.* 1994]. Excessive electrolyte and fluid secretion by crypt cells in combination with reduced numbers of absorptive villous cells would lead to diarrhoea which is often a symptom of coeliac disease. TGF-β, capable of inhibiting secretion and promoting epithelial barrier function [Howe *et al.* 2002], is under-expressed in coeliac disease [Lionetti *et al.* 1999; Hansson *et al.* 2002] and a number of transcription factors known to regulate this cytokine were found in this study. HFE, a gene involved in regulating iron transport and located in chromosome 6p21 (CELIAC locus 1) was found to be up-regulated in this study. Over-expression of HFE leads to reduced iron uptake [Roy and Enns 2000], another known event in coeliac disease, indicating that sensory signals involved in regulating transport may be skewed. Alterations in transcellular transport are thought to occur in coeliac disease [Matysiak-Budnik *et al.* 2003] and this may be how gliadin crosses the epithelial barrier to initiate the disease.

In conclusion, this study found a number of genes to have altered expression in active coeliac disease. These genes are involved in a number of processes, some of which are known to occur to a certain extent in coeliac disease and others which have not been previously reported. The application of microarray technology to the study of a complex genetic disease such as coeliac disease is of great use in the discovery and clarification of gene expression patterns, and could help in the elucidation of the pathogenesis of this disease.

4.1 Introduction

Having performed DNA microarray studies to compare gene expression in epithelial cells from active coeliac disease to controls, it was necessary to follow up this work with confirmatory studies in order to validate the initial findings. It is important to verify that the observed changes are accurate and reproducible and not the result of problems inherent to the array technology. The success and quality of a gene profiling experiment depends upon coherent secondary and tertiary studies to determine the biological function of a number of candidate genes.

Post-array verification of results can be perfeormed by two main approaches: *in silico* analysis or independent laboratory-based methods. *In silico* analysis uses information available in the literature and in public expression databases to compare to array results. This method is more useful for studies on commonly reported diseases such as cancer where large datasets have been previously published and are available for comparison. Laboratory-based validation typically involves using the same samples that were studied in the initial array experiments and a method that also measures mRNA expression such as northern blots, semi-quantitative reverse transcription PCR (RT-PCR), real-time RT-PCR, ribonuclease protection assay and *in situ* hybridisation [Chuaqui *et al.* 2002].

Following independent verification of the array data, the universality of the results may be tested. This is to determine whether the expression profiles are a universal feature of the biological condition under study and is undertaken by analysis of a number of genes in a different study group, often examining the expression at the protein level. Examples of methods used for this analysis are: immunohistochemistry either on individual samples or on tissue microarrays, western blots and protein microarrays.

The criteria for selection of genes for follow-up analysis in this study was based primarily on statistical significance. Genes with a range of p-values were chosen in order to determine whether there is a correlation between statistical significance and ability to replicate results. In order to pursue genes with a potential importance in

coeliac disease pathogenesis, genes were further selected on the basis of having medium to high-range fold-values as well as having a functional role in relevant biological mechanisms such as cell adhesion and signalling, cell transport and metabolism. Furthermore, many of these genes have previously been shown to be expressed in the small intestine.

The aim of this study was to corroborate and follow up the findings of the microarray study of gene expression in active coeliac disease epithelium (chpt 3). Ten genes with a spread of p-values (0.0033-0.16), medium- to high-range fold-changes (1.8 - 4.5-fold) and with relevant biological functions were selected for validation by real-time RT-PCR in order to determine whether there is a correlation between statistical significance and ability to replicate results. Two of these genes were further analysed for protein expression by immunohistochemistry.

4.2 Materials and Methods

4.2.1 Patients

For protein validation, archival formalin-fixed, paraffin-embedded duodenal biopsies were obtained with ethical approval, granted by the Joint Ethics Committee Board of St James' Hospital and Tallaght Hospital, Dublin, Ireland. All biopsies were taken from the duodenum, section 2 (D2), with the exception of the peptic duodenitis, disease control patients whose biopsies were taken from the duodenum, section 1 (D1). The following patients were used:

Untreated coeliac disease (UCD) patients (n=10)

Corresponding biopsies from the five untreated coeliac patients from chapter 3 were examined. A further five untreated patients were studied, of whom one had a type 3a duodenal lesion (chpt1.2.2), two had type 3b and two had type 3c. These patients consisted of 3 males and 2 females with a mean age of 59.8 years (range 54- 65).

Treated coeliac disease (TCD) patients (n=10)

Biopsies from the latter five UCD patients described above, taken at a later date, while following treatment, were examined. Histology reports showed that two patients had recovered to normal duodenal histology (from type 3a and type 3c lesions) while three patients had recovered to type 3a lesions with only mild or variable villous blunting (from type 3b and type 3c lesions). These patients had been following a gluten-free diet for a mean of 7 years (range 4-11 years).

Five more TCD patients were also studied, three of whom had normal duodenal histology, one had a type 1 lesion and one had a type 3a lesion. The mean length of time they were following a gluten-free diet was 1.7 years (range 1-2 years). This patient group consisted of 3 females and 2 males with a mean age of 51.4 years (range 44 - 69).

Disease control patients (n=10)

The first group of disease control patients consisted of five patients with Crohn's disease involving the duodenum. These patients consisted of 2 females and 3 males with a mean age of 50.8 years (30-79). The second group of disease control patients

consisted of five patients with peptic duodenitis. These patients consisted of 2 females and 3 males with a mean age of 43.4 (35-56).

Normal control patients (n=10)

Ten patients undergoing endoscopy for investigation of upper gastrointestinal symptoms were studied as a control group. All patients had normal duodenal histology. The group consisted of 6 females and 4 males with a mean age of 59.3 years (range 26-89).

For RT-PCR validation, the same patient samples obtained in section 3.2.1 were used. The stored total RNA (section 3.2.5) was utilised for this experiment.

4.2.2 Reverse transcription

300ng of total RNA was diluted to 5µl in sterile, deionised water and heated in a water-bath at 85°C for two minutes. The sample was then placed on ice for 1 minute. A master mix containing 5mM $MgCl_2$, avian myeloblastosis virus reaction buffer (50mM Tris-HCl pH 8.3, 50mM KCl, 10mM $MgCl_2$, 10mM DTT, 0.5mM spermidine), 1U/µl RNasin® ribonuclease inhibitor, 1U/µl avian myeloblastosis virus reverse transcriptase, 5ng/µl random primers, 1mM deoxynucleotide triphosphates (Promega, USA) was made up and 15µl was added to each denatured RNA sample. The reaction was performed in a hot-lid thermal cycler at 42°C for one hour. cDNA samples were diluted to 240µl in sterile, deionised water and were stored at -80°C.

4.2.3 Quantification of samples

cDNA samples were quantified using the Fluorescent DNA Quantitation Kit (Bio-Rad, UK). A standard curve was set up using the provided calf thymus DNA at quantities of 1000ng, 500ng, 200ng, 100ng, 50ng, 20ng and 10ng. Standards and samples (10µl) were loaded in triplicate into a 96-well micro-plate and 200µl of Hoechst 33258 dye (2 µg/ml) was added to each well. A blank containing only dye was also included in triplicate. The fluorescence was measured on a Tecan GENios Fluorimeter (Tecan, UK) using an excitation filter of 360nm and an emission filter of 465nm. The associated software, Xfluor, provided a read out of the plate in an Excel spreadsheet. A

calibration curve was created by plotting total DNA concentration (ng) versus relative fluorescence units (RFU).

4.2.4 TaqMan Primers and probes

Genes to be validated were selected on the basis of significance and potential interest. The genes were chosen to represent a spread of p-values up to 0.1 (table 4.2.1). Assays-on-Demand™ Gene Expression products (Applied Biosystems, UK) containing forward primer, reverse primer and probe labelled with FAM dye and MGB quencher in a single tube were used. These primer/probe mixtures were pre-designed by the manufacturer and pre-optimised to ensure high amplification efficiency (proprietary sequences).

Table 4.2.1 Genes examined by RT-PCR

Gene Name	p-value from microarray
sterol regulatory element binding transcription factor 1 (SREBF1)	0.003
syntaxin 3A (STX3A)	0.012
Laminin 5, alpha 3 (LAMA3)	0.030
peroxisome biogenesis factor 13 (PEX13)	0.039
nudix (nucleoside diphosphate linked moiety X)-type motif 2 (NUDT2)	0.043
Small proline-rich protein 3 (SPRR3 aka Esophagin)	0.051
sialic acid binding Ig-like lectin 6 (SIGLEC6)	0.056
gap junction protein, alpha 4, (GJA4 aka connexin 37)	0.066
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (HMGCS1)	0.074
low density lipoprotein receptor-related protein 6 (LRP6)	0.094

aka: also known as

4.2.5 Synthesis of standards

4.2.5.1 Preparation of cells

The Caco-2 cell-line (ECACC, UK), derived from a human colorectal adenocarcinoma, was used to create standards for the primers. Cells were cultured in DMEM culture media (GibcoBRL) with 10% fetal calf serum and 1% L-Glutamine-Penicillin-Streptomycin Solution (Sigma, UK) for 3 days until confluent. The supernatant was poured off and fresh media containing interferon-gamma (1000U) was added on top of the cells. The cells were incubated for four hours after which time, the supernatant was decanted and the cells washed with 10mls PBS. The PBS was poured off: 2mls trypsin/EDTA (GibcoBRL) was added and allowed to incubate at 37°C for 10 minutes. 10mls of preheated media (37°C) was added to inactivate the trypsin. The cells were then counted using Trypan Blue dye (1:5 dilution) in a haemocytometer. Viable cells stained white while non-viable cells appeared blue. The cell suspension was split into two aliquots and centrifuged at 12,000 rpm for 10 minutes to obtain a cell pellet.

4.2.5.2 RNA extraction

Total RNA was extracted using the NucleoSpin® RNA II kit (BD Biosciences Clontech, UK) as described in section 3.2.5, except the RNA eluate from the column was not subjected to further concentration. RNA was quantified and analysed as described before.

4.2.5.3 Reverse transcription

Aliqouts of RNA (5µl) were heated at 85°C for 2min and placed on ice. A reaction mixture containing 5mM $MgCl_2$, avian myeloblastosis virus reaction buffer (50mM Tris-HCl pH 8.3, 50mM KCl, 10mM $MgCl_2$, 10mM DTT, 0.5mM spermidine), 1U/µl RNasin® ribonuclease inhibitor, 1U/µl avian myeloblastosis virus reverse transcriptase, 5ng/µl random primers, 1mM deoxynucleotide triphosphates (Promega, USA) was added to the denatured RNA in a final volume of 20µl. The reaction was performed at 42°C for 60 minutes and cDNA samples were stored at -80°C.

4.2.5.4 Polymerase chain reaction (PCR)

The cDNA was PCR amplified using each of the ten primers. Five microliters of cDNA sample was added to a reaction mix of 25µl 2X SYBR® Green JumpStart™ Taq ReadyMix™ (20mM Tris-HCl, pH 8.3; 100mM KCl; 7mM MgCl2, 0.4 mM each dNTP, stabilizers, 0.05unit/µl Taq DNA Polymerase, JumpStart Taq antibody, SYBR Green I) (Sigma, USA), 2.5µl 20X primer/probe mix (Assays-on-Demand™, Applied Biosystems, UK) and 17.5µl deionised water. The PCR was carried out in a thermal cycler (PTC-100, Bio-Rad USA) using the settings described in appendix II-D.

4.2.5.5 Gel purification of PCR products

PCR products were purified using the QIAquick® Gel Extraction Kit (Qiagen Ltd, UK). Products were run on a 1.2% agarose/EtBr gel in 1X TBE buffer. Under UV light, PCR products were visualised as bands and a gel slice containing the band cut out. Each gel slice was weighed in a pre-weighed eppendorf tube. 300µl of buffer QG was added for each 100mg of gel and incubated at 50°C until the gel slice was completely dissolved. 100µl of isopropanol was added for each 100mg of gel and mixed by vortexing. The sample was loaded onto a QIAquick column and centrifuged at 13000 rpm for 1 minute. Flowthrough was discarded and 500µl Buffer QG was added onto the column in order to remove all traces of agarose. The column was centrifuged at 13000 rpm for one minute and flowthrough discarded. The sample was washed by addition of 750µl buffer PE onto the column and centrifugation at 13000 rpm for 1 minute. Residual ethanol from buffer PE was removed by an additional centrifugation step at 13000 rpm for one minute. The QIAquick column was placed in a fresh eppendorf tube and 50µl Buffer EB added onto the column to elute the DNA. The column was allowed to stand for one minute before centrifugation at 13000 rpm for 1 minute. Samples were run on a 1.2% agarose/EtBr gel in 1X TBE buffer and visualised under UV/Vis light to examine purity.

4.2.5.6 Quantification of standards

PCR products were quantified as described in section 4.2.3.

4.2.6 Absolute quantification by real-time PCR

The PCR products were used as standards [Øvstebø *et al.* 2003] to quantify mRNA expression, in the patient samples, by TaqMan RT-PCR.

4.2.6.1 Optimisation of standard curve

PCR products were diluted to a concentration of 1 x 10^3 pg/µl. A serial 10-fold dilution was performed down to a concentration of 1 x 10^{-7} pg/µl, and a number of standard curve tests were performed using varying concentrations and varying numbers of standards. The quality of the standard curve was assessed on the basis of the tightness of the replicates and the R^2 value of the trend-line created by the standards (the closer to 1, the better the fit for the data). The ability of the standards to reproduce a similar, good quality standard curve after one cycle of freeze-thawing was also examined.

4.2.6.2 .Optimisation of sample quantity

Due to limited amounts of sample available, samples were tested at a number of different quantities to find the minimum amount of sample required that would produce reliable and reproducible results.

4.2.6.3 Quantification of gene expression

Quantification of gene expression was carried out in the ABI TaqMan 7000 (Applied Biosystems). Each gene was quantified on a separate 96-well plate (MicroAmp® Optical 96-Well Reaction Plate, Applied Biosystems) containing the standards for that gene and all cDNA samples. A 10µl reaction volume consisting of 1X primer/probe mix (Assays-on-Demand™ Gene Expression products), 1X Universal PCR Master Mix (PCR buffer, deoxynucleotide triphosphates, AmpliTaq Gold, uracil-N-glycosylase) (Applied Biosystems), water and 2µl of standard, sample or water (as negative control) was loaded into each well. The reaction was carried out using the settings described in appendix II-A.

The ABI Prism 7000 SDS software (Applied Biosystems) was used to analyse the quantification. As part of the plate set-up before each reaction, the task of each well

was assigned and where the task was standard, the quantity could be assigned. After the amplification process was complete and a threshold cycle (C_T) (section 2.27) chosen, the software automatically created a standard curve with a best-fit trend line. The gene expression quantities of each sample were automatically calculated from this curve.

In order to compare the expression values to those obtained from the microarray analysis, the mean expression value of the five coeliac samples was divided by the mean expression value of the five control samples to give a single ratio value.

4.2.7 Antibodies

Rabbit polyclonal anti-human SPRR3 (small proline-rich protein 3) (Apotech UK), was employed at a concentration of 1 in 1000. Oesophageal tissue was stained as a control and used to optimise the staining conditions [Kimos *et al.* 2004]. Rabbit polyclonal anti-mouse connexin 37 (Alpha Diagnostic, US) was utilised at a concentration of 1 in 150. The mouse connexin 37 immunogenic peptide has an 87% homology to human connexin 37. Umbilical cord tissue was stained as a control and used in the optimisation of staining conditions [Van Rijen *et al.* 1997].

4.2.8 Immunohistochemical staining

Immunohistochemical staining was performed on 3μm-thick sections, cut from formalin-fixed, paraffin-embedded biopsies, using the avidin-biotin-peroxidase complex detection method. Sections were deparaffinized in xylene (2x5mins) and rehydrated in 100% industrial methylated spirit (IMS) (2x5mins), 95% IMS (5mins), distilled water (5mins) and TBS (5mins). For connexin 37 staining, antigen retrieval was carried out on the sections by immersion in citrate buffer (10mM sodium citrate, pH 6) and heating in a microwave oven for 25mins, followed by 20mins cooling at RT and 5mins cooling under running cold water. For SPRR3 staining, no antigen retrieval was necessary. Endogenous peroxidase activity was blocked by incubation in 1.5% hydrogen peroxide in methanol for 10mins. The staining procedure was carried out using the VECTASTAIN® Universal Elite ABC-Peroxidase Kit (Vector Labs, USA). Sections were incubated with blocking serum for 30mins at RT in a humidified staining tray, after which time excess serum was blotted away. The primary antibodies were diluted (as described in section 4.2.7) in TBS and added onto the sections which

were then incubated overnight at 4°C in a humidified staining tray. Excess antibody was removed by washing in TBS/Tween (3x3mins). Biotinylated secondary antibody was added onto the sections and allowed to incubate for 30mins at RT in a humidified staining tray, followed by washing to remove excess antibody as before. Sections were then incubated with VECTASTAIN® Elite ABC Reagent (Avidin/Biotinylated-peroxidase) for 30 min at RT in a humidified chamber, and washed as before. Substrate solution, diaminobenzidine tetrahydrochloride (DAB) was added to the sections for approximately 10mins and rinsed off with distilled water. The sections were counterstained with haematoxylin solution for 3mins and "blued" under running tepid water for 3mins, followed by immersion in distilled water. Dehydration through 95% IMS, 100% IMS x 2, and xylene x 2 (all for 5mins) was carried out, and the slides were then mounted using DPX solution and allowed to air-dry.

4.2.9 Analysis of staining

Slides were visualised using a Nikon eclipse TE300 microscope attached to a computer. Images were captured using Leica DC100 and Adobe Photoshop software.

4.3 Results

4.3.1 Quantification of cDNA samples

cDNA samples were quantified using the Fluorescent DNA Quantitation Kit (Bio-Rad, UK) and the Tecan GENios fluorimeter (Tecan, UK). Using the generated absorbance values for the standards, a calibration curve was created (Fig. 4.3.1). The concentration of the cDNA samples was determined using the least squares regression equation for the line generated by the standards, which is $y = mx + b$, where:

y is the instrument reading (RFU), x is the DNA concentration (ng), m is the slope of the line, and b is the y-intercept. The calculated concentration was then adjusted to account for the dilution factor and divided by the volume of sample used (10µl). The sample values are listed in table 4.3.1.

4.3.2 Purified PCR products

PCR products were purified using a gel extraction purification method. The purified DNA was analysed by agarose gel electrophoresis (Fig. 4.3.2).

4.3.3 Quantification of standards

Standard quantities were calculated as described in section 4.3.1. The concentration was determined in ng/µl and the values for each gene standard are listed in table 4.3.2.

4.3.4 Absolute quantification by real-time RT-PCR

4.3.4.1 Optimisation of standard curve

The standard curve was optimised using varying concentrations of standard and varying numbers of standard per curve. The best curve was found with 5 standards, whose concentrations were $1x10^{-1}$ pg/µl, $1x10^{-3}$ pg/µl, $1x10^{-5}$ pg/µl, $1x10^{-6}$ pg/µl and $1x10^{-7}$ pg/µl, where R_2=0.997551 (Fig. 4.3.3).

4.3.4.2 Optimisation of sample quantity

The minimum sample quantity that produced reliable results was 250pg per reaction. Samples were diluted to a concentration of 125pg/µl so that 2µl of sample could be added to each reaction well.

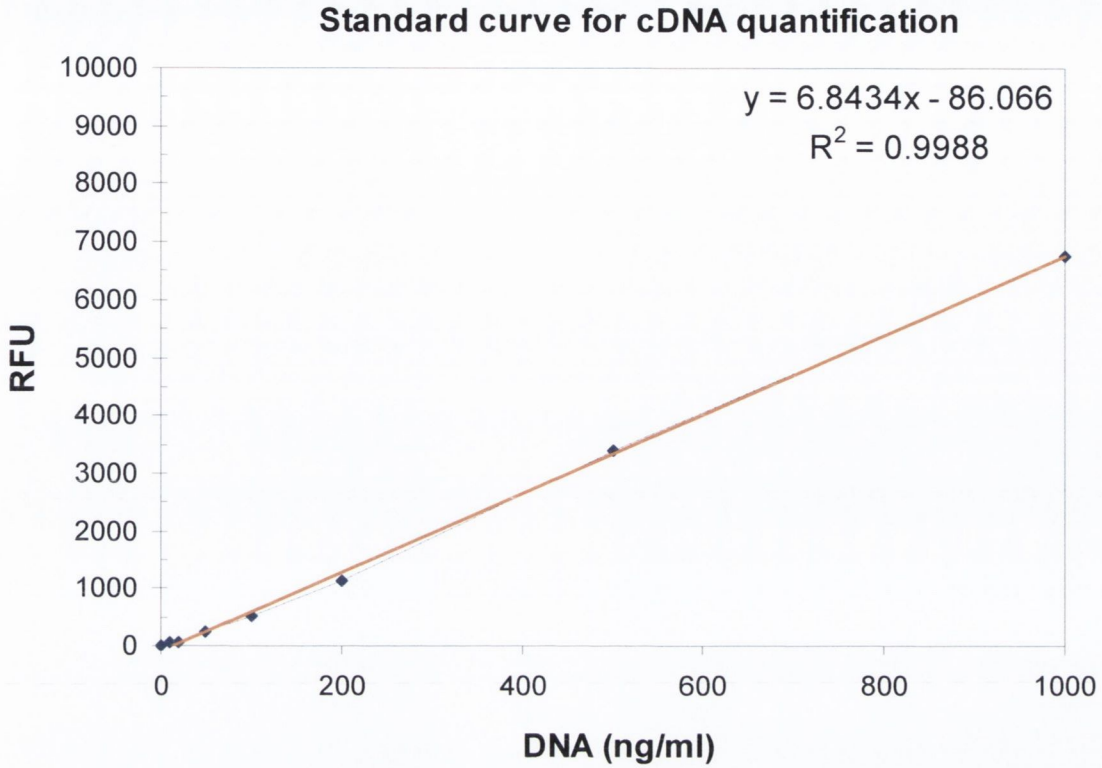


Figure 4.3.1: Standard curve used to quantify cDNA samples.

Sample No.	Ave OD	Calculated Conc. (from graph)	cDNA (ng/ml)	cDNA (pg/µl)
Coeliac 1	72.33	23.40	467.94	467.94
Coeliac 2	99.33	27.34	546.76	546.76
Coeliac 3	30.33	17.27	345.34	345.34
Coeliac 4	132.33	32.15	643.09	643.09
Coeliac 5	67.3333	22.67	20.00	453.35
Control 1	34.33	17.85	357.02	357.02
Control 2	61.33	21.79	435.83	435.83
Control 3	43.3333	19.16	20.00	383.29
Control 4	142.3333	33.61	20.00	672.28
Control 5	62.33	21.94	438.75	438.75

Table 4.3.1: cDNA sample quantities calculated from standard curve.



Figure 4.3.2: Purified PCR products used as standards for TaqMan quantification of gene expression.

Sample Name	Ave OD	Calculated Conc. (from graph)	DNA (ng/ml)	DNA (ng/ul)
SIGLEC6	654.0	46.59	9364.08	9.36
NUDT2	490.0	29.69	5967.37	5.97
HMGCS1	1471.0	130.77	26285.46	26.29
LAMA3	1518.0	135.62	27258.90	27.26
LRP6	1144.0	97.08	19512.76	19.51
SPRR3	1028.0	85.13	17110.22	17.11
GJA4	537.0	34.53	6940.82	6.94
SREBF1	459.0	26.58	5341.80	5.34
STX3A	514.0	32.13	6459.06	6.46
PEX13	444.0	24.99	5022.47	5.02

Table 4.3.2: Standard (PCR product) quantities calculated from standard curve.

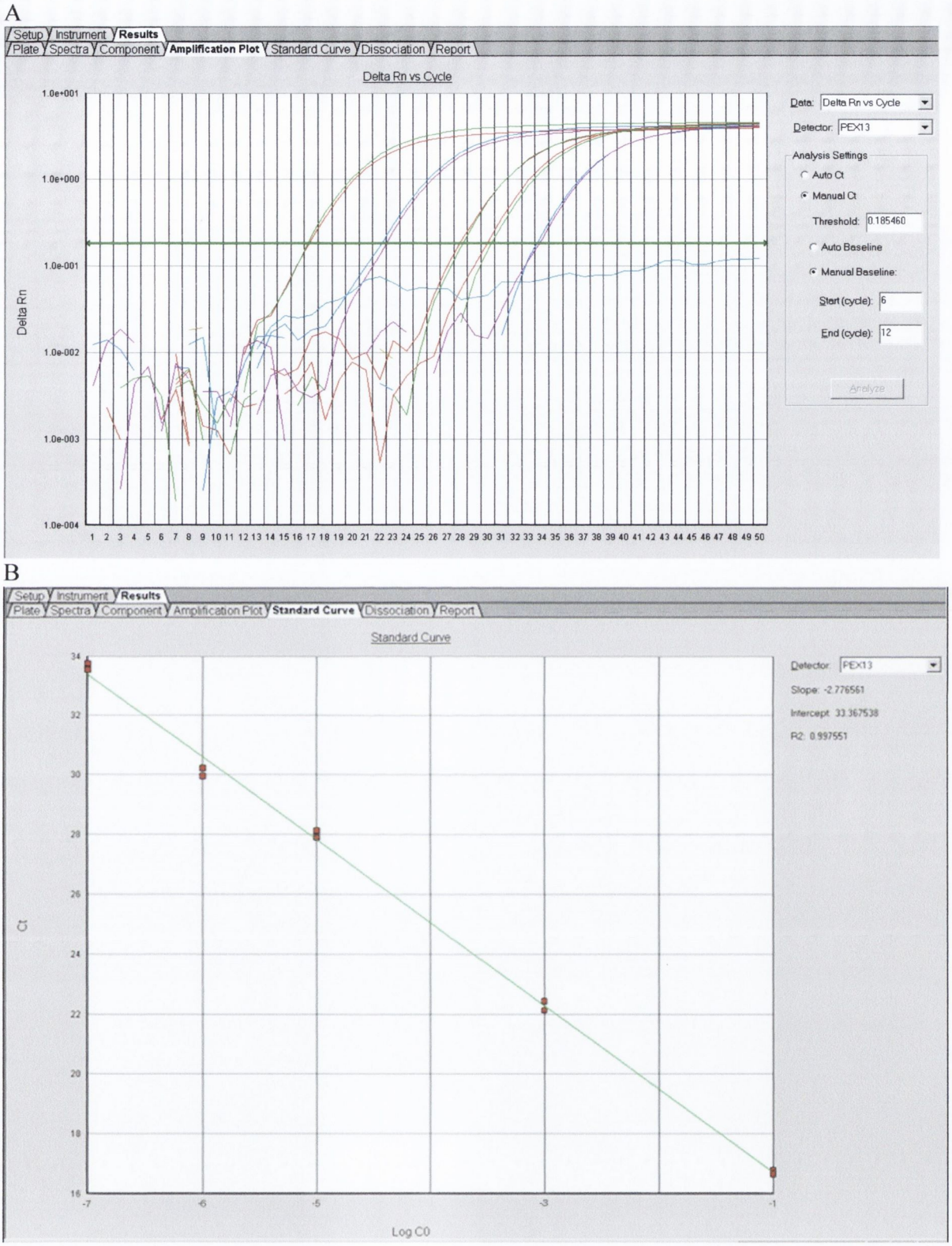


Figure 4.3.3: (A) Amplification plot of standards (serial dilutions) and (B) standard curve plotting the Ct values of the standards against the dilution factor (expressed in log).

4.3.4.3 Quantification of gene expression

In order to corroborate the microarray gene expression results, ten genes were selected for validation by real-time RT-PCR quantification. The real-time RT-PCR expression values were calculated from a standard curve in the form of pg $x10^n$, where n is the unit plotted on the graph (Fig. 4.3.3). The mean of the values calculated for the coeliac samples was divided by the mean for the control samples to give a ratio.

4.3.5 Comparison of real-time RT-PCR and microarray results

The ratios calculated above were then compared to the microarray ratios (table 4.3.3). Eight of the ten genes showed up-regulation of expression in coeliac disease with both microarray and real-time RT-PCR analysis. One gene showed up-regulation of expression in coeliac disease with microarray analysis and non-differential expression with real-time RT-PCR. One gene showed down-regulation of expression in coeliac disease with microarray analysis and up-regulation of expression with real-time RT-PCR analysis.

4.3.6 Analysis of staining

Two of the genes validated by real-time RT-PCR, were selected for examination of protein expression. Qualitative analysis was performed on small proline-rich protein 3 and gap junction protein, alpha 4 (aka connexin 37).

Small proline-rich protein 3:

Optimisation of staining was performed using oesophageal tissue. Positive staining of the stratified squamous epithelium was observed (Fig.4.3.4a and b) in a pattern comparable to previous reports, where intense cytoplasmic and peripheral staining of the epithelial cells was demonstrated [Hohl *et al.* 1995; Kimos *et al.* 2004]. A negative control, using TBS buffer in place of the primary antibody, was carried out and figures 4.3.4c and d demonstrate no non-specific staining was seen in this section.

Protein expression of small proline-rich protein 3 (SPRR3) was examined in biopsies from five untreated coeliac patients and in corresponding biopsies taken from these same patients at a later date while consuming a gluten-free diet. In the untreated coeliac tissue the common finding was intense cytoplasmic staining of SPRR3 throughout the epithelial cell, with strongest staining observed on the apical side of the

Gene	TaqMan rt-PCR fold-change	Microarray fold-change	P-value from microarray
peroxisome biogenesis factor 13 (PEX13)	1.62	2.28	0.0033
gap junction protein, alpha 4, (GJA4, aka connexin 37)	4.09	1.95	0.0058
syntaxin 3A (STX3A)	1.52	1.81	0.0058
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (HMGCS1)	1.25	0.54	0.0068
sterol regulatory element binding transcription factor 1 (SREBF1)	1.26	4.53	0.0354
nudix (nucleoside diphosphate linked moiety X)-type motif 2 (NUDT2)	3.02	2.21	0.0370
Small proline-rich protein 3 (SPRR3, aka Esophagin)	1.77	2.1	0.0398
sialic acid binding Ig-like lectin 6 (SIGLEC6)	3.14	1.89	0.0982
Laminin 5, alpha 3 (LAMA3)	1.94	2.51	0.0993
low density lipoprotein receptor-related protein 6 (LRP6)	1.04	2.12	0.160

Table 4.3.3: Comparison of fold-changes obtained for genes analysed by microarray and TaqMan RT-PCR.

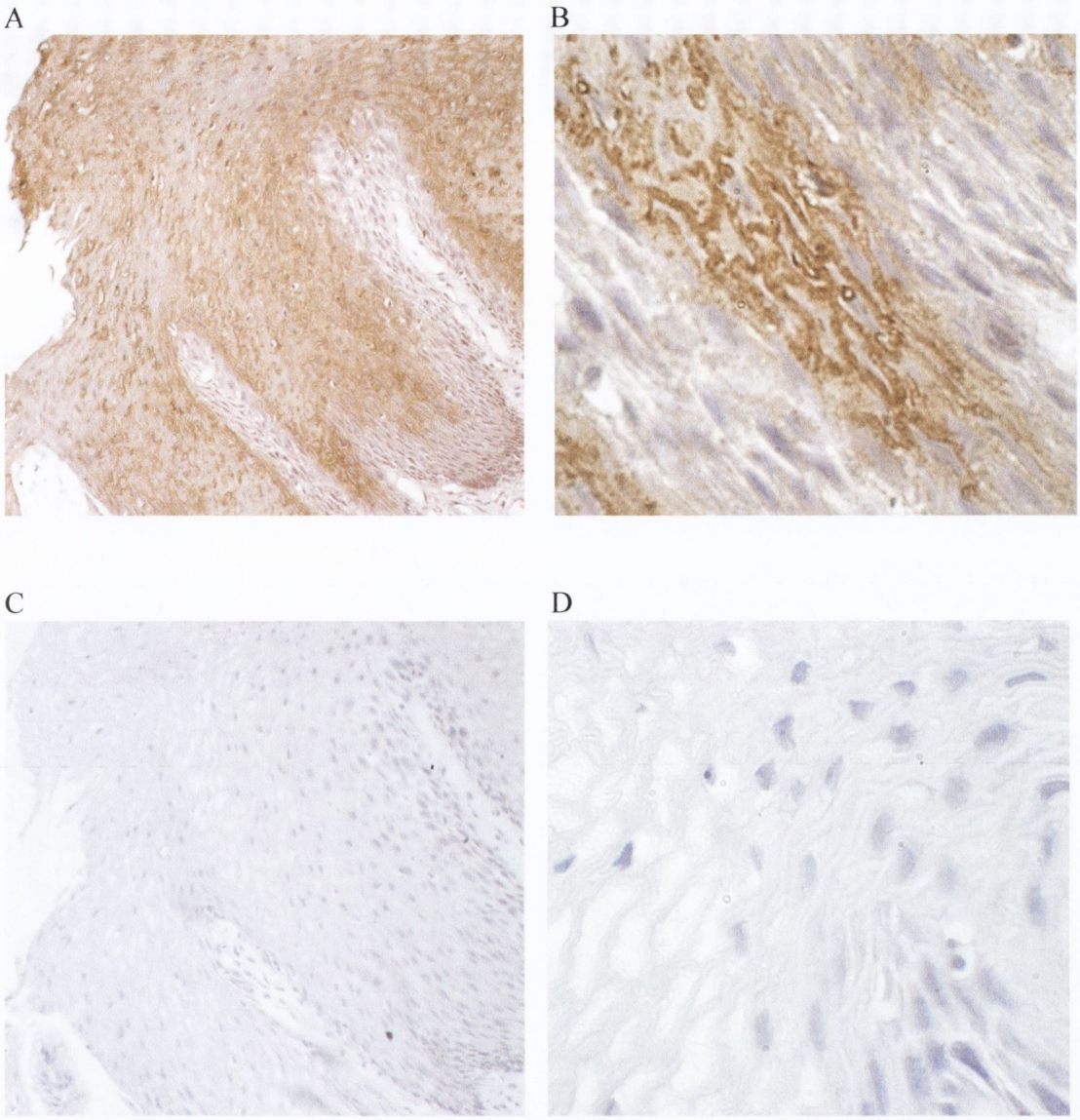


Figure 4.3.4: Immunohistochemical detection of SPRR3 in oesophageal tissue. A and B are positive controls, visualised at 20x and 100x respectively. Intense cytoplasmic staining of the squamous epithelial cells is observed. C and D are negative controls, visualised at 20x and 100x magnification respectively. No staining of SPRR3 is observed.

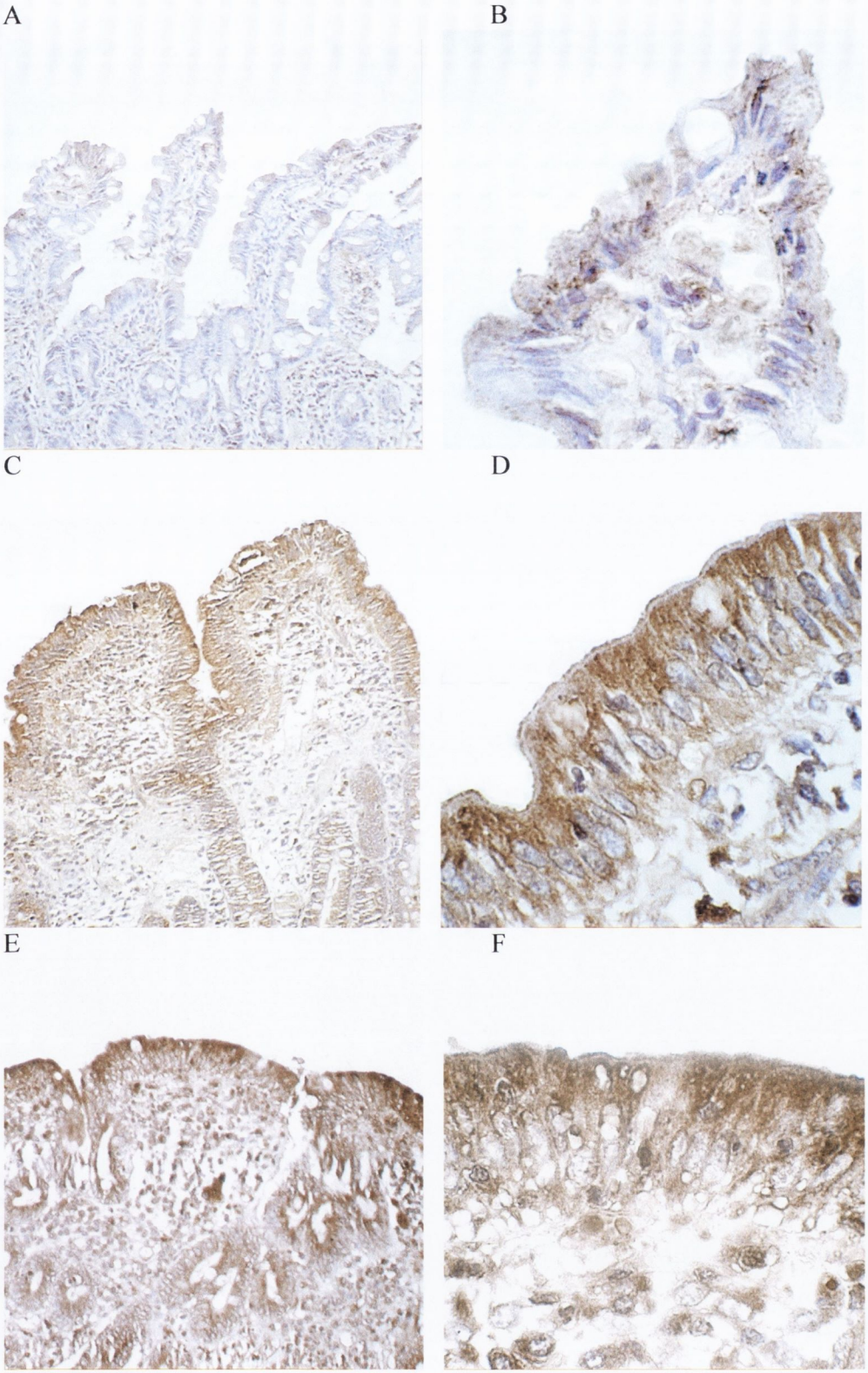


Figure 4.3.5: Immunohistochemical detection of SPRR3 in duodenal tissue. A, C and E are at 20x magnification, B, D and F are at 100x magnification. A & B: treated coeliac, showing faint staining in villous-tip epithelial cells, with occasional punctate staining of moderate intensity; C & D: untreated coeliac (same patient as A and B) and E & F: untreated coeliac (patient 2 from microarray study, chapter 3), showing intense cytoplasmic staining throughout epithelial cells with strongest staining observed on apical side of cell.

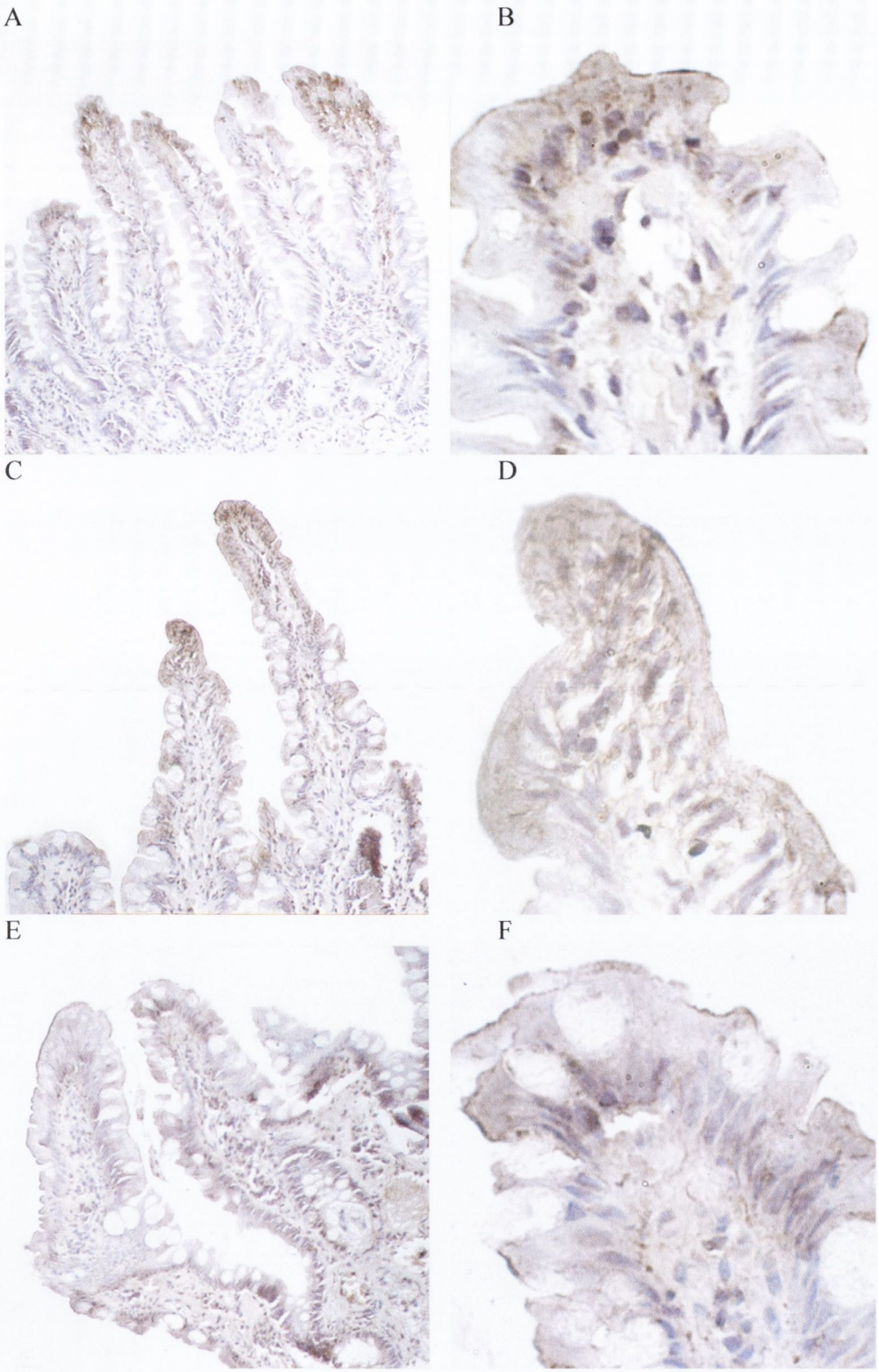


Figure 4.3.6: Immunohistochemical detection of SPRR3 in duodenal tissue. A, C and E are at 20x magnification, B, D and F are at 100x magnification. A and B: normal control; C and D: disease control (duodenal Crohn's); E and F: disease control (peptic duodenitis). All samples showed minimal staining of epithelium with only villous-tip involved.

cell (Fig.4.3.5c and d). Perinuclear staining was also detected although less visible due to the intensity of the cytoplasmic staining. The staining was observed all along the epithelium with the top part of the villus showing the greatest intensity. Comparing the untreated biopsies to the treated, up-regulation of expression was found in four out of the five patients, with approximately equal expression observed in the fifth pair of biopsies. The treated coeliac tissue generally showed faint staining in the villous-tip epithelial cells, with occasional punctate staining of moderate intensity also occurring only in the villous-tip region (Fig.4.3.5a and b).

Biopsies from the five previously-studied (chpt.3) untreated coeliac patients who showed up-regulation of SPRR3 mRNA expression by microarray and RT-PCR analysis were also examined for protein expression of SPRR3. All five samples showed a similar intense staining picture as seen in the untreated coeliac biopsies described above (Fig. 4.3.5e and f).

Biopsies from ten normal control patients and ten disease control patients- consisting of five with peptic duodenitis and five with Crohn's disease with a duodenal involvement- were examined for SPRR3 protein expression. Similar to the picture seen with the treated coeliac samples, all control samples showed minimal staining of the epithelium with only the villous-tip involved (Fig 4.3.6).

SPRR3 staining of the stroma was also evident but appeared to be non-specific in nature. Artifactual staining was likely caused by the poor quality of the tissue which appears to have broken up at places.

Gap junction protein, alpha 4 (Connexin 37):

Staining for gap junction, alpha 4 (GJA4) was optimised using umbilical cord. The endothelial cells of the umbilical cord artery stained positively (Fig 4.3.7a and b), as has been previously reported [Van Rijen *et al.* 1997]. TBS buffer was used in place of the primary antibody as a negative control and no non-specific staining was seen in this section (Fig 4.3.7c and d).

Expression of GJA4 protein was compared between the matched pairs of untreated and treated coeliac biopsies. In the untreated epithelial tissue, granular staining was predominantly seen in the cytoplasm of the epithelial cells, with an increasing intensity towards the brush border (Fig 4.3.8c and d). Perinuclear staining was visible in some cells although nuclear staining was generally absent. In contrast, in the treated

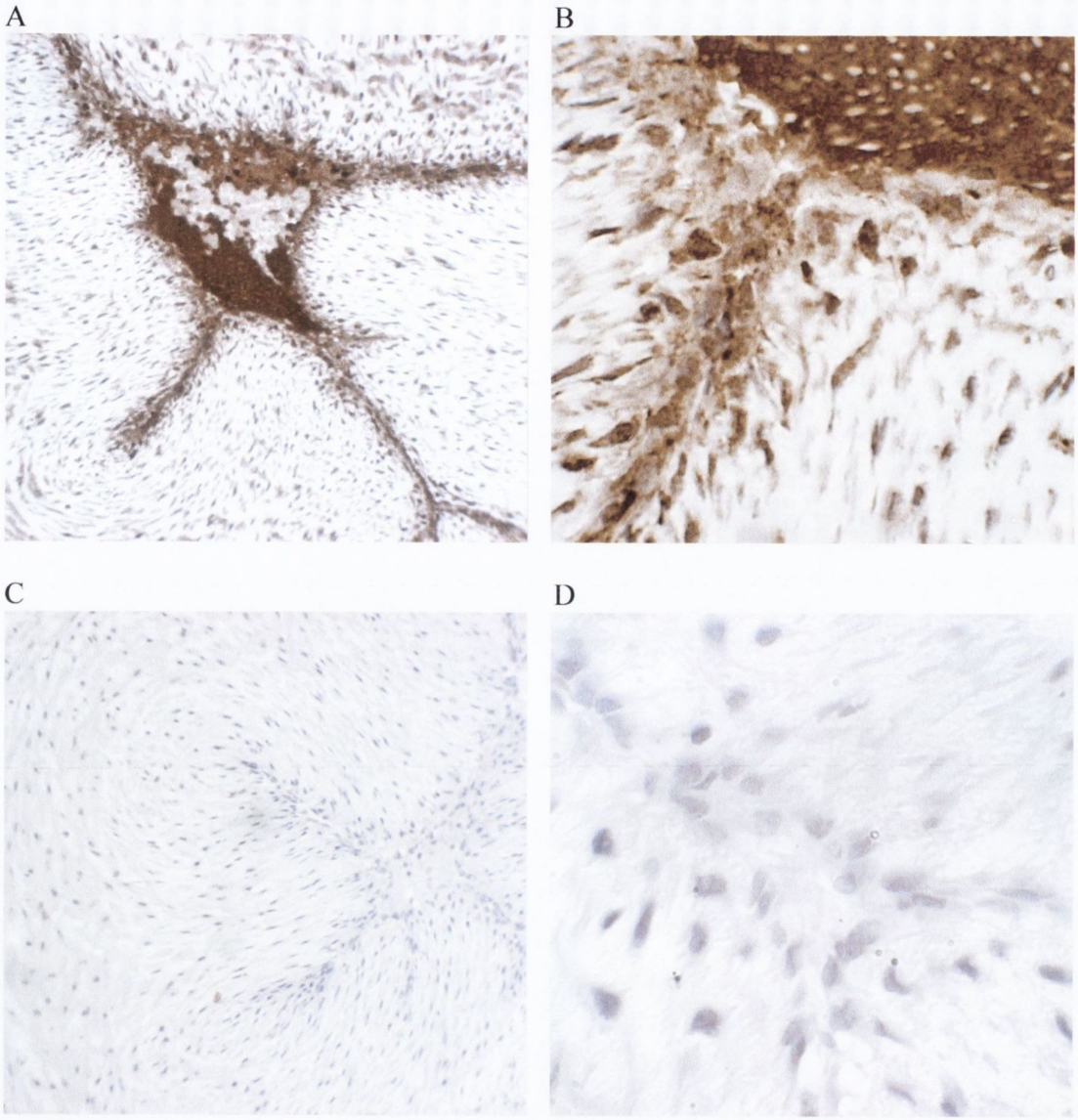


Figure 4.3.7: Immunohistochemical detection of GJA4 in umbilical cord tissue. A and B are positive controls, visualised at 20x and 100x respectively. Intense cytoplasmic staining of the endothelial cells is observed. C and D are negative controls, visualised at 20x and 100x magnification respectively. No staining of GJA4 is observed.

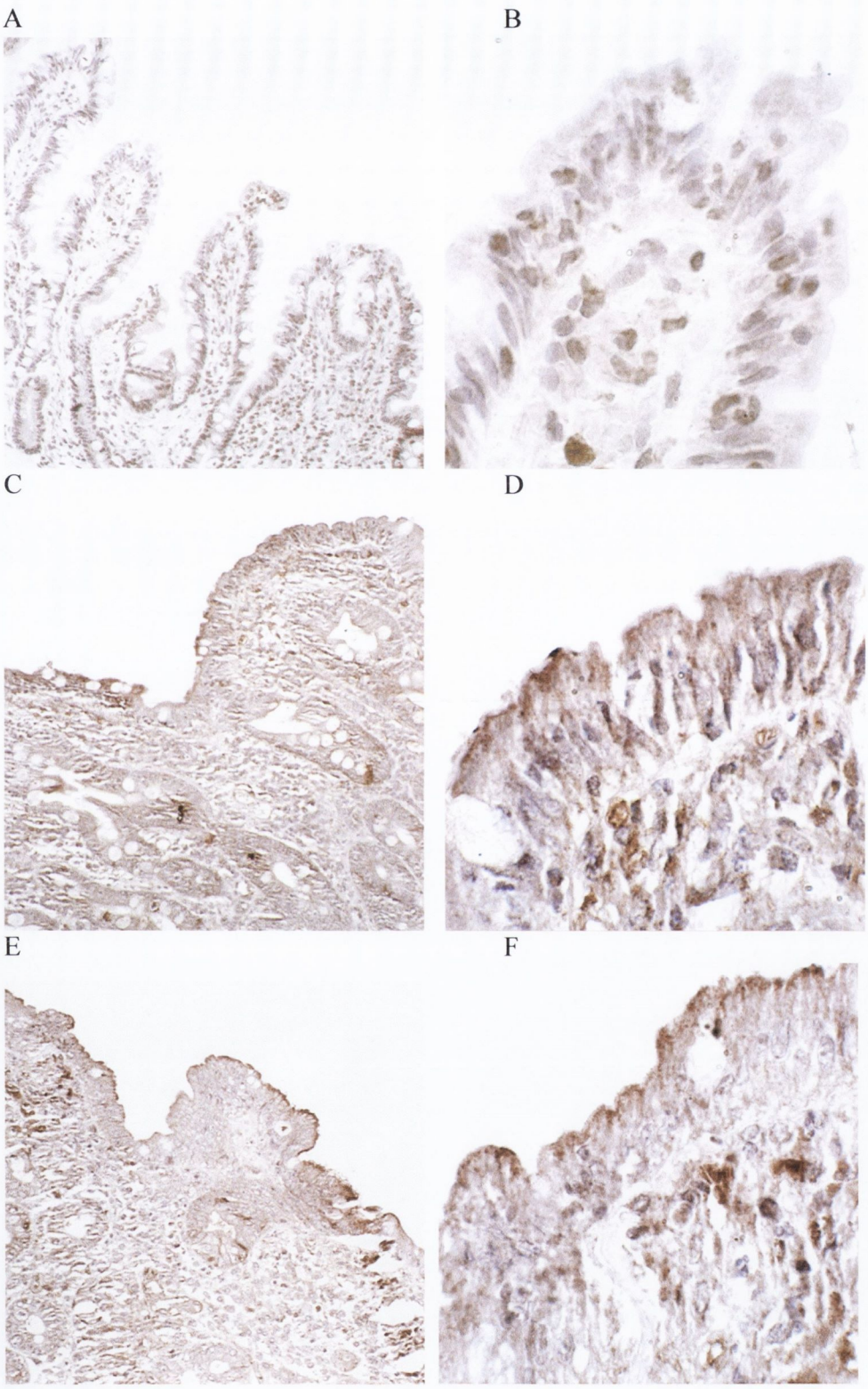


Figure 4.3.8: Immunohistochemical detection of GJA4 in duodenal tissue. A, C and E are at 20x magnification, B, D and F are at 100x magnification. A & B: treated coeliac, nuclear staining of epithelial cells apparent while brush border staining absent; C & D: untreated coeliac (same patient as A and B) and E & F: untreated coeliac (patient 1 from microarray study, chapter 3), granular staining predominantly seen in cytoplasm of epithelial cells with increasing intensity towards brush border. Perinuclear staining visible in some cells although nuclear staining generally absent.

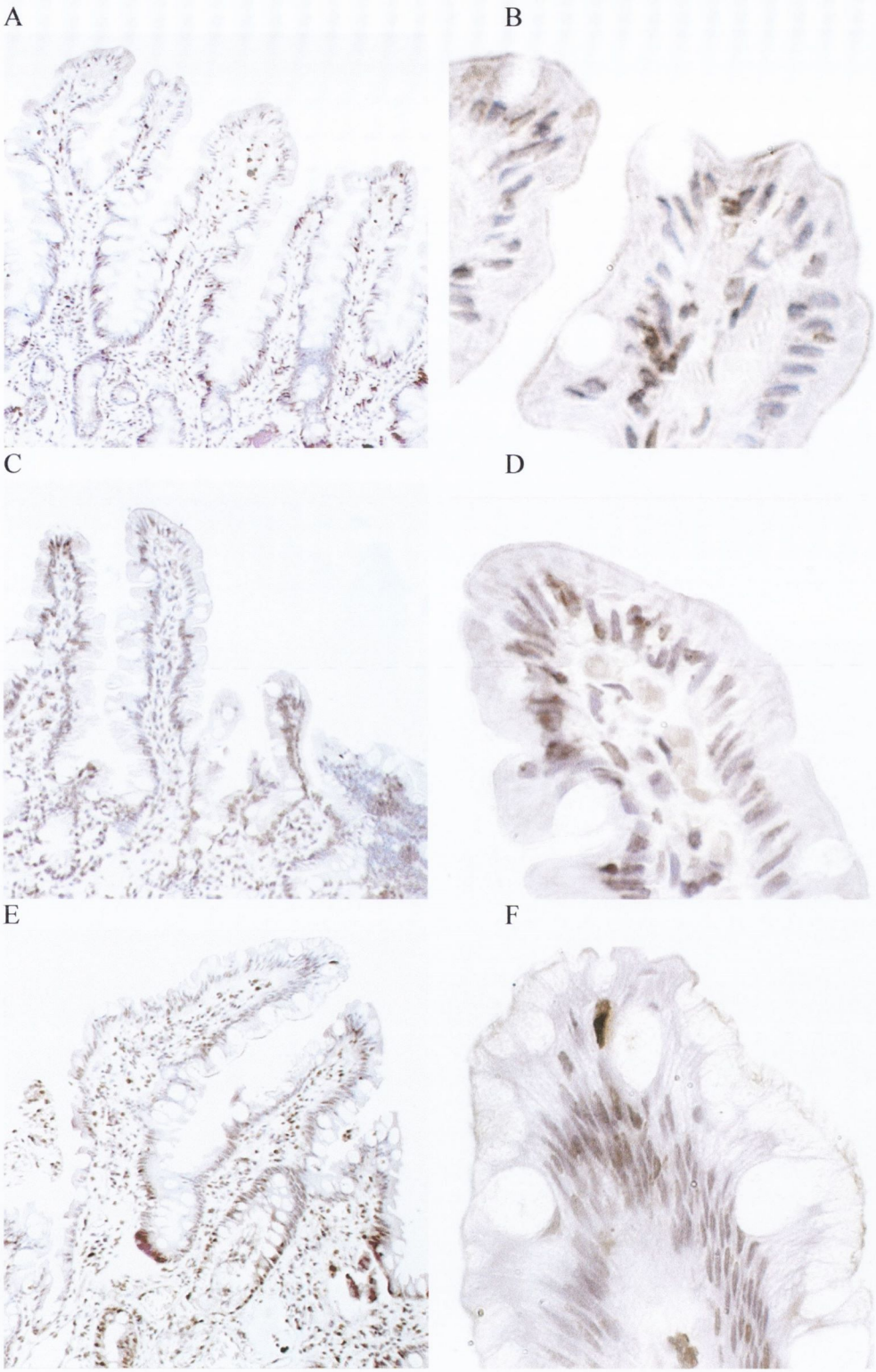


Figure 4.3.9: Immunohistochemical detection of GJA4 in duodenal tissue. A, C and E are at 20x magnification, B, D and F are at 100x magnification. A and B: normal control; C and D: disease control (duodenal Crohn's); E and F: disease control (peptic duodenitis). All samples showed perinuclear staining in the epithelium, similar to treated coeliac tissue, but with less frequent nuclear staining.

biopsies, nuclear staining of epithelial cells was common while weak cytoplasmic staining was only evident in the villi tips and brush border staining was absent. The intra-epithelial lymphocytes within the treated tissue showed the most intense nuclear staining while the staining of enterocytes appeared to be due to strong perinuclear staining (Fig 4.3.8a and b).

In biopsies from the five previously-studied (chpt. 3) untreated coeliac patients, who showed up-regulation of GJA4 mRNA expression by microarray and RT-PCR analysis, staining was similar to that seen in the untreated coeliac biopsies described above (Fig.4.3.8e and f).

Biopsies from control patients (Fig 4.3.9), both normal and diseased, showed a similar staining pattern to that observed in treated coeliac tissue although less cells appeared to stain positively in the control tissues. In particular, nuclear staining was less frequent than in the treated coeliac tissue while perinuclear staining remained a common finding.

GJA4 staining was also seen in the stroma of all patient tissue types and was slightly increased in the treated and untreated coeliac tissue compared to the normal and disease control tissue. Nuclear staining of the lamina propria lymphocytes and macrophages appear to be the main source of this staining.

4.4 Discussion

The purpose of this study was to authenticate and further investigate the differential gene expression found in active coeliac disease by microarray analysis. Validation experiments were carried out to assess the accuracy and reliability of the microarray results. Following this, two genes were further investigated at the protein level to evaluate their contribution to the disease process.

Ten genes with a range in p-values from 0.0033 to 0.16 were analysed by real-time RT-PCR using the same patient samples from the microarray study. Eight of the ten genes showed differential gene expression in the same direction in both microarray and RT-PCR analysis. These genes that showed up-regulation in both microarray and RT-PCR analysis were: sialic acid binding Ig-like lectin 6 (SIGLEC6), laminin 5, alpha 3 (LAMA3), syntaxin 3A (STX3), peroxisome biogenesis factor 13 (PEX13), nudix (nucleoside diphosphate linked moiety X)-type motif 2 (NUDT2), sterol regulatory element binding transcription factor 1 (SREBF1), small proline-rich protein 3 (SPRR3) and gap junction protein, alpha 4 (GJA4). The latter two genes are described in more detail later in the discussion.

Sialic acid binding Ig-like lectin 6 (SIGLEC6), also known as CD33L, is a member of a recently designated family of cell surface molecules which are a subset of I-type lectins and in turn, belong to the larger immunoglobulin superfamily [Patel *et al.* 1999]. Previously shown to be expressed in the small intestine, this molecule functions in cell adhesion and cell-cell signalling by interacting with sialylated glycoprotein ligands expressed on specific cell populations [Patel *et al.* 1999]. Siglec-6 has another alias, obesity-binding protein (OB-BP1), so named because it was found to bind the hormone leptin (a homolog of the mouse obesity gene) and it is hypothesised that Siglec-6 regulates circulating levels of leptin [Patel *et al.* 1999].

Laminin 5, alpha 3 (LAMA3) is a component of the basement membrane with a function in cell adhesion and signal transduction. In the normal small intestinal basement membrane, different laminin chains show differential distribution along the crypt-villous axis with laminin 5, alpha 3 chain expressed in the upper villous zone

[Korhonen *et al.* 2000]. TNF-alpha and IFN-gamma synergistically stimulate the secretion of laminin-5 by epithelial cells [Francoeur *et al.* 2004]. In Crohn's disease, where these proinflammatory cytokines are over-expressed, laminin 5, alpha 3 is found up-regulated in the crypt compartment as well as being expressed in the villus compartment [Bouatrouss *et al.* 2000]. LAMA3 has also been shown to be over-expressed in oral squamous cell carcinoma [Snijders 2005].

Syntaxin 3A (STX3) is a component of vesicle trafficking which functions in intracellular protein transport. It is a member of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein family which participate in the docking of vesicles to cell membranes. Syntaxin 3a is expressed apically in intestinal epithelial cells [Breuza *et al.* 2000] and is associated with tubulovesicles containing H,K-ATPase (proton pump) in gastric parietal cells [Karvar *et al.* 2005]. Syntaxin 3a has also been found to be up-regulated during kidney development [Lehtonen *et al.* 1999].

Peroxisome biogenesis factor 13 (PEX13) is also involved in intracellular protein transport, in particular with peroxisome matrix protein import. Peroxisomes are subcellular membrane-bound compartments with a function in cellular metabolism. They contribute to the ß- and α-oxidation of fatty acids, oxidation of bile acids and cholesterol and syntheses of ether-phospholipids [Maxwell *et al.* 2003]. Pex13 is a component of the peroxisomal docking complex which is involved in the import of matrix proteins into the peroxisome and mutations in this gene can lead to Zellweger syndrome, an often fatal, cerebrohepatorenal syndrome.

Nudix (nucleoside diphosphate linked moiety X)-type motif 2 (NUDT2) is a member of the MutT family of nucleotide pyrophosphatases, a subset of the larger NUDIX hydrolase family. These enzymes are believed to eliminate toxic nucleotide derivatives from the cell and regulate the levels of important signalling nucleotides and their metabolites. NUDT2 asymmetrically hydrolyzes Ap4A (diadenosine 5', 5'''-P^1, P^4-tetraphosphate) to yield AMP and ATP. Ap4A has been shown to induce apoptosis and cause a decrease in the level of Bcl-2 protein [Vartanian *et al.* 2003].

Sterol regulatory element binding transcription factor 1 (SREBF1) is a transcription factor that binds to the sterol regulatory element-1 and is involved in lipid and cholesterol metabolism. Insulin is known to induce expression of SREBF1 whereas polyunsaturated fatty acids have been shown to decrease expression of SREBF1 in intestinal epithelial cells [Field 2002]. SREBF1 is activated by oxidised phopholipids in atherosclerotic lesions and is involved in the induction of IL-8 [Yeh *et al.* 2004].

Eighty percent of the genes tested in this study were found to be validated which compares well to a reported average RT-PCR confirmation rate of 70% [Pusztai and Hess 2004]. Interestingly, the gene with the highest p-value (0.16) was not validated by RT-PCR which may imply that the less statistically significant the result is, the less reliable it is. However, another gene which did not validate had a low p-value (0.0068); thus, a linear correlation between statistical significance and variation of the results between the two methods was not found. Genes ranging from p-values with 0.0033 to 0.0993 were found to be validated with differing levels of variation between the two methods (Fig. 4.3.3). Differences in gene expression levels found between microarray and real-time RT-PCR analysis are commonly reported in the validation of microarray analysis, and genes are described as validated as long as differential expression in the same direction is shown in both methods [Rajeevan *et al.* 2001; Srisuma *et al.* 2003; Spira *et al.* 2003; Hong *et al.* 2004]. It is unsurprising that variation is found considering these are two different techniques. The oligonucleotides on the microarray, and the primers for RT-PCR likely target different regions within the gene and may hybridise to the cDNA with different efficiencies. The microarrays used in this study are made with long oligonucleotides (80 bases) which are considered to have greater sensitivity and less specificity than shorter oligonucleotides, such as those found on Affymetrix arrays (25 bases) [Relógio *et al.* 2002]. A lack of specificity could allow cross-hybridisation to occur, particularly amongst gene family members with a high degree of homology. Although the microarrays used in this study are considered to have good sensitivity, real-time RT-PCR is deemed to be the most sensitive and specific method of gene quantification currently available [Wong and Medrano 2005].

Two genes, found to be up-regulated in active coeliac disease by microarray and real-time RT-PCR, and with statistically significant fold-changes were selected for protein expression analysis by immunohistochemistry. These genes were also selected on the basis of their involvement in biological mechanisms relevant to the coeliac small intestinal lesion, these being structural and intercellular-transport functions. It should also be noted that selection of validated genes for follow-up protein analysis was limited to those with commercially available antibodies. Small proline-rich protein 3 (SPRR3) and gap junction protein, alpha 4 (GJA4) expression was examined in untreated and treated coeliac disease tissue as well as in normal and disease control tissue.

A distinct increase in the intensity of small proline-rich protein 3 (SPRR3) staining was found in the epithelium of untreated coeliac biopsies compared to the treated coeliac and control biopsies. This demonstrates that the increased mRNA transcription of SPRR3 in active coeliac disease leads to the translation into increased protein. The biopsies taken when the coeliac patients were following treatment with a gluten-free diet, show that the protein expression levels revert to that of normal tissue. This indicates that the increase in SPRR3 seen in untreated biopsies is a marker of active disease. The disease control biopsies, taken from patients with peptic duodenitis and with Crohn's disease in the duodenum, show no increase in protein levels compared to normal controls, implying that the increased protein found in untreated coeliac biopsies is not a general feature of duodenal inflammatory disease.

Small proline-rich protein 3 (SPRR3), also known as esophagin, is a structural protein and a member of the cornified cell envelope precursor family. Other structural proteins involved in the cornified cell envelope are desmoplakin, envoplakin, periplakin, annexin I, cystatin, trichohyalin, loricrin and involucrin [Steinert 1999]. The cornified cell envelope provides a vital physical barrier in certain specialised epithelia normally subjected to mechanical trauma. It is assembled by transglutaminase cross-linking of several proteins including SPRR3. SPRR3 has been shown to be a substrate for transglutaminase 1, 2 and 3, and is cross-linked at similar efficiencies by these enzymes [Steinert 1999]. Expression of SPRR3 is found in a variety of terminally differentiated epithelia such as oesophagus, filiform ridges of the tongue, inner root

sheath of the hair follicle and is up-regulated in response to epithelial injury or disease [Steinert 1999]. In normal skin epidermis, SPRR1 and SPRR2 are expressed while SPRR3 is absent; however, in differentiating epidermal tumours such as keratocanthoma and squamous cell carcinoma, SPRR3 expression is induced [De Heller-Milev *et al.* 2000]. The up-regulation of SPRR3 in active coeliac disease may be a defensive response to protect the mucosa from any further damage caused by the ingestion of gluten. The increased expression of transglutaminase 2 found in the enterocytes and basement membrane in active coeliac disease [Hansson *et al.* 2002; Esposito *et al.* 2003; Sakly *et al.* 2005] could cross-link with SPRR3 to form a cornified envelope-like barrier.

Gap junction protein, alpha 4 (GJA4) protein expression was differentially expressed between untreated and treated coeliac tissue. The intensity of the staining in the brush-border region of the untreated coeliac biopsies was increased compared to the treated coeliac biopsies. On the other hand, increased nuclear staining was evident in the epithelium of treated biopsies compared to untreated. This finding demonstrates that GJA4 protein expression is altered during the progression of coeliac disease Biopsies from disease control patients did not show the same pattern of protein expression as found in untreated coeliac biopsies, thus implying the altered protein expression of the untreated coeliac epithelial tissue is not a general feature of duodenal inflammatory disease. Both the normal and disease control epithelial tissue showed a pattern of GJA4 protein expression similar to treated coeliac epithelial tissue but appeared to have less frequency of nuclear staining. Biopsies taken from coeliac patients while following treatment on a gluten-free diet often demonstrate that the increased intra-epithelial lymphocyte (IELs) numbers of active disease do not fully revert to that of normal tissue even. This remaining increase in IELs in the treated coeliac tissue may account for the increase in frequency of cells with nuclear staining of GJA4 found in this tissue. An increase in the amount of staining was observed when comparing the untreated coeliac epithelial tissue to that of the normal controls. GJA4 mRNA was found to be up-regulated in active coeliac disease compared to normal controls and it is likely that this up-regulation of mRNA leads to the observed alterations in protein expression.

Gap junction protein, alpha 4 (GJA4), also known as connexin 37, is a member of the connexin family of gap junction structural proteins. Hexameric assemblies of these proteins, termed connexons, in the cell membrane of one cell interact with connexons of an adjacent cell to form intercellular channels. These gap junction channels are relatively non-specific and allow passive diffusion of small molecules up to 1000 daltons [Flagg-Newton *et al.* 1979]. Gap junctions play an important role in intercellular communication and allow the passage of ions, second messengers (such as Ca^{2+}, IP3, cAMP, and cGMP) and small metabolites [Seul *et al.* 2004]. In developmental or physiological situations, gap junctional intercellular communication can play various roles in terms of cell proliferation, migration and differentiation [Nishimura *et al.* 2004]. For example, blocking connexin 43 expression resulted in a reduction of cell proliferation and stunting of facial primordia outgrowth [McGonnell *et al.* 2001]. Similarly, retinoic acid-induced differentiation of tracheo-bronchial epithelial cells inhibited proliferation and reduced connexin 32 expression [Albright 2002]. Connexin 37 (GJA4) as well as connexins 40 and 43 is known to be expressed in vascular endothelium, although variations in expression occur in different vessels and under different physiological conditions [Hill *et al.* 2002]. A polymorphism in connexin 37 has been found to have an association with coronary heart disease [Yamada *et al.* 2004]. In studies following the progression of atherosclerosis, connexin 37 was shown to be expressed in the endothelium of non-diseased aortas while in advanced atheromas, connexin 37 was detected in medial smooth muscle cells and in macrophages in the lipid core but not in the endothelium covering the plaques [Kwak *et al.* 2002]. The alteration of expression of GJA4 from treated to untreated coeliac disease also displays changes in location of protein expression. Whether these changes in gap junction protein expression contribute to the disease progression in coeliac disease is unclear at this time.

The regulation of SPRR3 and GJA4 protein expression in active coeliac disease appeared to follow that of mRNA expression, implying that the increased levels of mRNA are linked to the increased levels of protein. Although the differences in GJA4 staining pattern in the various tissues made it more difficult to assess, overall an increased amount of staining in the untreated coeliac epithelial tissue was observed compared to normal controls. It is often noted that mRNA regulation does not

necessarily correlate to protein regulation [Chuaqui *et al.* 2002; Chen 2002]; nevertheless, it appears in this study that there was a correlation. However, as immunohistochemistry is not a quantitative technique, a direct comparison cannot be definitively made. Another protein analysis method, western blotting, could be used to measure the total expression of a given protein within a cell population but as this method is only semi-quantitative and provides no information on the localisation or distribution of the protein, it would not provide better information.

In conclusion, this study demonstrated the validity of the results obtained from microarray analysis. Eighty percent of the genes examined were validated by RT-PCR, showing the likelihood of other genes found with similar p-values to be true results. Immunohistochemical analysis of two of these genes, SPRR3 and GJA4 showed that the high levels of mRNA observed with microarray analysis are translated into high levels of protein.

Chapter 5

General Discussion

An intolerance to gluten is the cause of coeliac disease and occurs in genetically predisposed persons. Gluten is the term given to the prolamin and glutelin storage proteins in cereal grains. The alcohol-soluble prolamin fraction is generally considered to be the main constituent causing the intolerance and has a different name in different cereals: this includes gliadin in wheat, hordein in barley, secalin in rye and avenin in oats. The response to gluten has been studied in numerous experimental models and much has been learnt about the pathogenic response in coeliac patients over the past 55 years. The focus of this thesis was to assess the molecular response to gluten stimulation in order to gain an understanding about how different forms of gluten affect the coeliac small intestinal mucosa and what processes are involved in the gluten-induced damage.

5.1 Summary of results.

In chapter two, duodenal biopsies were stimulated *ex vivo* with wheat, barley and rye prolamins and the production of cytokines interferon-gamma and interleukin-2 was quantified as a measure of T-cell activation. The results of this study showed that these three cereal prolamins induce an immune response in coeliac patients *ex vivo* with an increased production of IFN-γ mRNA but not of IL-2 mRNA. Wheat gliadin has previously been shown to induce an up-regulation of cytokine production [Kontakou *et al.* 1995; Nilsen *et al.* 1998; Westerholm-Ormio *et al.* 2002; Forsberg *et al.* 2002; Kilmartin *et al.* 2003] but this is the first study to show that barley and rye can elicit an increase in small-intestinal interferon-gamma production in coeliac patients. In contrast to interferon-gamma, interleukin-2 was not found to be increased following stimulation which is in keeping with previous findings in coeliac disease [Nilsen *et al.* 1998; Forsberg *et al.* 2002] and other conditions with chronic immune activation [Kusugami *et al.* 1991; Semmo *et al.* 2005]. These results concur with the limited scientific evidence that like wheat, barley and rye are also toxic to coeliac patients and agree with the practice of excluding barley and rye from the gluten-free diet.

The study in chapter two examines the cytokine production by T-helper-1 cells which is a known step in the adaptive immune response pathway in coeliac patients. In this pathway, intestinally-expressed transglutaminase-2 deamidates gluten peptides allowing them to bind to antigen-presenting cells expressing HLA-DQ2 or –DQ8. The

peptides are then presented to $CD4^+$ T-helper cells which become activated and release pro-inflammatory cytokines. The cytokines exert their effect either directly on intestinal epithelial cells or activate other cells such as macrophages to secrete damaging components such as matrix-metalloproteinases. This adaptive immune response pathway has been well studied and important information has been learnt [Sollid 2002; Cerf-Bensussan *et al.* 2003]. However, the contribution to this process of other major intestinal cells such as the epithelial cell is not well studied and how these cells respond or contribute to the gluten-induced inflammatory environment is not known. For this reason, experiments were set up to examine the molecular events in the epithelial cell following *in vivo* gluten stimulation. As it was not desired to restrict the study to known or assumed processes, a hypothesis-free approach was undertaken to analyse the molecular response.

Thus, in chapter three, a microarray study was performed to compare the gene expression profile in the duodenal epithelial cells of coeliac patients ingesting a gluten-containing diet to that of non-coeliac control patients also consuming a gluten-containing diet. A number of genes were found to have significantly altered expression in active coeliac disease. The main functional categories found represented amongst these genes were cell growth and/or maintenance, metabolism and development. Examination of genes with a cytokine function and genes found in the CELIAC loci, although often not reaching statistical significance, showed a regulation of genes involved in similar functions. The biological pathway in which the most altered genes participated was the apoptosis pathway while other biological pathways found to interact with this pathway and have a number of genes involved were focal adhesion and Wnt signalling. Transcription factor binding site analysis was performed on the significantly altered genes and a number of *cis*-regulatory elements were identified. The functional analysis revealed a number of common themes. Firstly, proliferation would seem to be increased while differentiation decreased; and secondly, survival appeared to be increased with apoptosis decreased.

It is essential to follow up microarray gene expression analysis with confirmatory studies in order to assess the accuracy and reliability of the results. A follow-up study may also be performed to ascertain whether the expression profiles are a universal

feature of the biological condition under study, in this case, coeliac disease. This is achieved by analysis of a number of genes in a different study group, often examining the expression at the protein level. The goal of any molecular study is ultimately to determine if a protein has altered expression and if this alteration leads to a biological consequence.

In chapter four, ten genes from the microarray study with a spread of statistical p-values were selected for validation by real-time RT-PCR in order to determine whether there is a correlation between statistical significance and ability to replicate results. Two of these genes were further analysed for protein expression by immunohistochemistry. Eighty percent of the genes examined were validated by RT-PCR (compared to the reported average RT-PCR confirmation rate of 70% [Pusztai and Hess 2004]), demonstrating the high quality of the microarray system used. Immunohistochemical analysis of two of these genes, SPRR3 and GJA4 showed that the high levels of mRNA observed with microarray analysis were translated into high levels of protein. From this study, it can be concluded that the majority of results obtained by microarray analysis are likely to be true results and can reveal alterations in expression that cause an affect at the protein level.

5.2 Innate immunity in coeliac disease.

In recent years, many researchers have begun to suspect that the innate immune system may have more of a role to play in the coeliac response to gluten than previously thought. In particular, the question continually asked is how do gluten peptides breach the epithelium to initiate the adaptive immune response pathway. An increase in intestinal permeability, in theory, could be facilitated by a transient infection which would lead to the activation of antigen-presenting cells and T-cells and, thus, the production of proinflammatory cytokines. Types I and II interferons which are released during infection can increase epithelial permeability [Kagnoff 2005]. Evidence for increased permeability in active coeliac disease comes from the observation that zonulin (a zonula occludens toxin that causes the opening of intestinal tight junctions leading to increased paracellular permeability) is up-regulated in active coeliac disease [Fasano *et al.* 2000]. However, the inhibitory effect upon tight junction protein zonula

occludens has been shown to be reversed after a gluten-free diet, ruling out a constitutive defect in coeliac patients [Pizzuti *et al.* 2004].

In the microarray study performed in this thesis, many genes involved in transport were found to be regulated in active coeliac disease. Examples of such genes are syntaxin 3a (STX3A), gap junction protein, alpha 4 (GJA4) and peroxisome biogenesis factor 13 (PEX13) and some of these genes may contribute to the transport of gluten peptides in coeliac disease. Alternatively, the alteration in intestinal transport in active coeliac disease may be a result of the immaturity of the villus epithelial cells generated by the hyper-proliferative crypt compartment. Crypt hyperplasia occurs first in the architectural changes during the development of the coeliac intestinal lesion [Oberhuber *et al.*1999], a factor often overlooked in the discussion of the pathogenesis of coeliac disease. Therefore, it seems likely that the initial event in the disease process is the deregulation of processes in the crypt. This point argues against the theory that activation of T-cells leading to villous atrophy is the main explanation to the structural alterations in the intestinal mucosa.

This thesis demonstrates regulation of gene expression that would lead to an increase in proliferation and survival of epithelial cells in coeliac disease; and highlights a number of biological pathways which have become altered, for example, the Wnt signalling pathway, known to be involved in the regulation of intestinal epithelial progenitor cells [Sancho *et al.* 2004]. A genetic defect in the regulation of these processes may be involved in the development of coeliac disease. Of all complex genetic diseases, coeliac disease has one of the highest concordance rates (75%) amongst monozygotic twins and the HLA region is known to only partially contribute to this rate. The remaining contributory genes have yet to be elucidated. Many of the genes shown in this thesis to have an altered expression in active coeliac disease could be further investigated and may provide hypotheses for future directions of research in coeliac disease.

5.3 Future directions.

Microarray analysis and expression profiling holds great promise for the exploration of many research avenues in coeliac disease. Chapter two of this thesis demonstrates that cereals other than wheat cause immune activation in coeliac disease; yet this work also highlights the difficulties in studying cereal prolamin responses, perhaps explaining why there is a dearth of scientific evidence on this subject. Comparing microarray expression profiles of coeliac tissue in response to different prolamins, or in response to different peptides within a single prolamin, would provide a means to gaining new information in this area.

Gene association and linkage studies are likely to play an important role in the future of coeliac disease research. Candidate gene association studies have so far been restricted to genes with known or potential immunological functions, within the CELIAC susceptibility loci. This approach has not yet had success in finding a susceptibility gene and it is likely that genes with unknown relevance may confer susceptibility to coeliac disease. This was recently shown to be the case in rheumatoid arthritis where a variant in an organic cation transporter gene, SLC22A4, was discovered to be associated with rheumatoid arthritis in the Japanese population [Tokuhiro *et al.* 2003]. Therefore, a hypothesis-free approach to selecting genes for coeliac genomic studies may have more success. Microarray gene expression studies highlight genes that are transcriptionally regulated differently between the disease and control population and thus, could be useful in prioritising genes in the future for gene association studies.

From the microarray study performed in this thesis, it can be seen that a number of genes within important biological pathways were differentially expressed in active coeliac disease. An interesting future direction could be to study the contribution of these pathways to the pathogenesis of the disease. RNA interference- a new molecular technique in which the introduction of small fragments of double-stranded RNA whose sequence matches a given gene interferes with the expression of that gene causing it to be silenced- could be used to determine the role of a given gene within a pathway or to assess the importance of a particular pathway within the disease process.

Future research is likely to involve many modern techniques that are currently being developed. RNA interference (RNAi) is emerging as a powerful tool for genetic analysis and is rapidly being applied to study the function of many genes associated with human disease. Currently, there is an intense research effort aimed at developing short interfering RNAs for therapeutic purposes and a number of proof-of-principle experiments have demonstrated the clinical potential of appropriately designed short interfering RNAs in various diseases. Examples of these experiments are: a study which demonstrated sustained siRNA-mediated inhibition of HIV type 1 in terminally differentiated macrophages, which constitute an important reservoir of HIV *in vivo* [Song *et al.* 2003]; and, a cancer study where RNAi was targeted against, and reduced the expression of the oncogenic fusion gene BCR/ABL mRNA in cells from patients with chronic myeloid leukaemia [Scherr *et al.* 2003]. Most recently, Sirna-027 (Sirna Therapeutics, Inc), an siRNA drug which targets the vascular endothelial growth factor (VEGF) pathway, has reached phase 1 clinical trials in the US for the treatment of age-related macular degeneration. At present, the major limitations to short RNA-mediated therapy are inefficient *in vivo* delivery and uncertainty about potential toxicity [Hannon and Rossi 2004]. Solutions to these obstacles are being worked on and it is likely that with further research, RNAi will have a prevalent role in the management of various human diseases.

DNA microarrays are likely to feature commonly in future research as the technology continually advances and the cost reduces. There are still challenges that need to be overcome such as the need for standardisation of array manufacturing techniques, assay protocols and analytical methods used to interpret data. Resolving these issues will allow microarray research to offer an increased ability to unravel complex disease processes and determine new targets for therapeutic interventions. The first microarray-based diagnostic test, Roche's AmpliChip CYP450 Test, was cleared by the US Food and Drug Administration (FDA) in January 2005. The test detects variations in a patient's cytochrome P450 2D6 and 2C19 genes, which play a primary role in the metabolism of many drugs including anti-depressants, anti-psychotics, anti-arrhythmics, beta-blockers, anti-convulsants, proton pump inhibitors and some anti-cancer drugs. The purpose of the test is to provide a predicted phenotype, that is, whether the patient is a poor, intermediate, extensive or ultra-rapid metaboliser.

From the advances in DNA microarrays, other forms of microarrays have evolved. The tissue microarray consists of an array of cylindrical cores of paraffin-embedded tissue that are removed from multiple pre-existing paraffin blocks. A morphologically representative area of interest within the original block is initially identified using a hematoxylin and eosin stained section as a guide. The tissue cores are removed from the original block and inserted into a new paraffin block usually using a tissue arrayer such as those produced by Beecher Instruments, Inc. Using a precise spacing pattern, tissues are inserted at high density, with up to 1000 tissue cores in a single paraffin block. Sections from this block are cut and placed onto standard slides that can then be used for *in situ* analysis. Depending on the overall depth of the original tissue, tissue arrays can generate between 100 and 500 sections. Once constructed, tissue microarrays can be used with a wide range of techniques including immunohistochemical/immunofluorescent staining and *in situ* hybridisation for either DNA or mRNA. One area where tissue microarrays are being used frequently is in the validation of DNA microarrays [Shergill *et al.* 2004] where the high throughput nature of the tissue array allows for faster and more economical analysis of targets than conventional histology methods. Clinical applications of this technology are in the screening for biomarkers, assessment of tumour or disease progression and in predicting patient prognosis [Kalliomieni *et al.* 2001]. One potential limitation of tissue microarrays is that the small cores sampled may not be representative of the whole tissue. This issue has been critically evaluated and it has been proposed that using three to four cores from the original biopsy accurately represents the tissue sample [Rubin *et al.* 2002].

Protein microarrays are another form of microarray technology that has been recently developed to aid in the study of protein interactions and function. These arrays can be divided into two main categories: capture arrays and interaction arrays. Capture arrays have immobilised molecules such as antibodies or chemically treated surfaces, which bind to target proteins. Interaction arrays have immobilised biological molecules such as proteins or peptides, which are used to identify functions or determine interactions with other proteins or small molecules. Many applications of protein arrays have been demonstrated, including auto-antibody profiling: Robinson *et al.* [2002] created auto-antigen arrays which comprised proteins, peptides, enzyme complexes,

ribonucleoprotein complexes, DNA and post-translationally modified antigens which were used to characterise sera from patients with autoimmune diseases including systemic lupus erythematous and rheumatoid arthritis. Another example of an application of protein arrays is cancer research: Sun *et al.* [2004] manufactured a protein microarray with monoclonal antibodies against a number of tumour markers and screened serum samples from cancer patients as well as from asymptomatic patients from high-risk populations. A further application of protein arrays is in drug discovery: for example, Lee *et al.* [2004], developed an integrin protein microarray which was applied for the high-throughput library screening of new active peptides against a specific integrin-ECM (integrin $\alpha_v\beta_3$-vitronectin) interaction. Protein microarrays pose a significant set of analytical challenges not faced by gene arrays such as the lack of availability of high quality, specific antibodies or suitable protein binding ligands, the conservation of protein functionality during immobilisation, and the provision of the required absolute and relative sensitivity [Angenendt 2005]. Despite these current limitations to the technology, protein microarrays hold great promise for the future as both a research and diagnostic tool.

Coeliac disease is currently the autoimmune disorder where the relationships between environment and genetics are best understood [Koning *et al.* 2005]. The environmental trigger, gluten; the autoantigen, tissue transglutaminase; and the predisposing HLA molecules, DQ2 or DQ8 are all known, and in recent years, many important insights into the complicated disease process have been discovered. With the continuous development and optimisation of new and exciting molecular techniques, the complete elucidation of the pathogenesis of coeliac disease is surely possible in the foreseeable future.

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Appendices

APPENDIX I

Flow cytometer settings used in chapter 3

Detector	Voltage	Amp Gain	Mode	Threshold	Compensation
FSC	E00	1.31	Lin	54	
SSC	394	2.09	Lin	52	
FL1	472	1.00	Log	52	4.9% FL2
FL2	492	1.00	Log	52	18.6% FL1

APPENDIX II

A. TaqMan (ABI 7000) settings

Carryover decomtamination via UNG	50°C	:	2 minutes
DNA polymerase pre-activation	95°C	:	10 minutes
40 cycles of:			
Denaturation	95°C	:	15 seconds
Annealing/Extension	60°C	:	60 seconds

B. PCR (PTC-100) settings used in section 3.2.8

Initial denaturation	95°C	:	1 minute
X cycles of:			
Denaturation	95°C	:	5 seconds
Annealing	65°C	:	5 seconds
Extension	68°C	:	3 minutes

C. PCR (PTC-100) settings used in section 3.2.9

Initial denaturation	95°C	:	5 minutes
3 cycles of:			
	94°C	:	1 minute
	25°C	:	1 minute, 30 seconds
	25-50°C:		increasing 0.1°C/second
	50°C	:	10 minutes
	68°C	:	5 minutes

D. PCR (PTC-100) settings used in section 4.2.5.4

Initial denaturation	95°C	:	5 minutes
45 cycles of:			
Denaturation	95°C	:	15 seconds
Annealing	60°C	:	1 minute
Extension	72°C	:	1 minute

APPENDIX III

Normalised gene expression results from chapter 3

Gene Name	normalised fold-change (range)	t-test p-value
2,3-bisphosphoglycerate mutase	1.439 (1.17 to 1.769)	0.329
2'-5'-oligoadenylate synthetase 3, 100kDa	1.075 (1.029 to 1.123)	0.347
3-hydroxy-3-methylglutaryl-Coenzyme A reductase	0.925 (0.763 to 1.12)	0.753
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	0.535 (0.431 to 0.68)	
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	0.839 (0.444 to 1.39)	0.514
3-hydroxysteroid epimerase	1.384 (0.829 to 2.465)	0.255
3-oxoacid CoA transferase	2.357 (0.908 to 12.66)	0.24
3-phosphoinositide dependent protein kinase-1	1.447 (1.023 to 1.906)	0.181
4-hydroxyphenylpyruvate dioxygenase	1.021 (0.686 to 1.363)	0.904
5,10-methylenetetrahydrofolate reductase (NADPH)	0.827 (0.72 to 0.95)	0.402
5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	1.175 (0.965 to 1.659)	0.45
5-hydroxytryptamine (serotonin) receptor 1A	2.651 (0.834 to 8.423)	0.554
5-hydroxytryptamine (serotonin) receptor 1B	1.261 (0.902 to 1.924)	0.408
5-hydroxytryptamine (serotonin) receptor 1D	1.026 (0.618 to 1.546)	0.9
5-hydroxytryptamine (serotonin) receptor 1E	1.291 (1.201 to 1.387)	0.175
5-hydroxytryptamine (serotonin) receptor 2B	1.142 (0.677 to 1.615)	0.667
5-hydroxytryptamine (serotonin) receptor 2C	1.396 (0.866 to 2.424)	0.22
5-hydroxytryptamine (serotonin) receptor 3A	1.016 (0.819 to 1.158)	0.853
5-hydroxytryptamine (serotonin) receptor 6	1.174 (0.886 to 1.843)	0.255
5-methyltetrahydrofolate-homocysteine methyltransferase reductase	0.957 (0.749 to 1.224)	0.888
5'-nucleotidase, ecto (CD73)	0.774 (0.486 to 1.037)	0.389
6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	0.95 (0.882 to 1.023)	0.615
6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2	1.67 (0.99 to 5.452)	0.287
6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	0.989 (0.434 to 2.648)	0.974
6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	0.648 (0.634 to 0.662)	0.032
6-pyruvoyltetrahydropterin synthase	1.098 (0.923 to 1.497)	0.456
6-pyruvoyl-tetrahydropterin synthase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1)	1.222 (0.826 to 1.609)	0.291
7-dehydrocholesterol reductase	1.012 (0.777 to 1.333)	0.948
8-oxoguanine DNA glycosylase	1.276 (0.783 to 3.562)	0.466
a disintegrin and metalloproteinase domain 10	0.634 (0.412 to 0.811)	0.169
a disintegrin and metalloproteinase domain 11	1.002 (0.851 to 1.18)	0.992
a disintegrin and metalloproteinase domain 12 (meltrin alpha)	0.823 (0.523 to 1.628)	0.493
a disintegrin and metalloproteinase domain 15 (metargidin)	0.978 (0.652 to 1.401)	0.894
a disintegrin and metalloproteinase domain 17 (tumor necrosis factor, alpha, converting enzyme)	0.885 (0.767 to 1.021)	0.55
a disintegrin and metalloproteinase domain 2 (fertilin beta)	0.89 (0.844 to 0.938)	0.271
a disintegrin and metalloproteinase domain 22	0.799 (0.333 to 1.423)	0.521
a disintegrin and metalloproteinase domain 23	0.951 (0.922 to 0.98)	0.347
a disintegrin and metalloproteinase domain 8	1.357 (0.95 to 1.937)	0.549
a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1	1.057 (0.464 to 2.407)	0.957
a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 4	0.832 (0.819 to 0.845)	0.0543
a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (aggrecanase-2)	1.106 (0.876 to 1.397)	0.74
a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 8	0.604 (0.494 to 0.738)	0.241
A kinase (PRKA) anchor protein (gravin) 12	0.9 (0.898 to 0.901)	0.00942
A kinase (PRKA) anchor protein (yotiao) 9	0.558 (0.153 to 1.129)	0.464
A kinase (PRKA) anchor protein 1	0.748 (0.74 to 0.757)	0.0248
A kinase (PRKA) anchor protein 10	1.408 (1 to 2.711)	0.406
A kinase (PRKA) anchor protein 13	1.603 (1 to 2.57)	0.5
A kinase (PRKA) anchor protein 2	0.797 (0.588 to 1.081)	0.593
A kinase (PRKA) anchor protein 3	0.766 (0.7 to 0.838)	0.207
A kinase (PRKA) anchor protein 4	1.497 (0.817 to 3.862)	0.347
A kinase (PRKA) anchor protein 5	1.065 (0.997 to 1.139)	0.516
A kinase (PRKA) anchor protein 6	0.901 (0.872 to 0.932)	0.197
A kinase (PRKA) anchor protein 7	0.473 (0.131 to 1.151)	0.373

A kinase (PRKA) anchor protein 8	0.405	no replicates
abl-interactor 2	0.985 (0.932 to 1.04)	0.825
absent in melanoma 2	1.07 (0.999 to 1.146)	0.506
acetylcholinesterase (YT blood group)	1.288 (0.684 to 1.927)	0.337
acetyl-Coenzyme A acetyltransferase 1 (acetoacetyl Coenzyme A thiolase)	0.833 (0.755 to 0.92)	0.316
acetyl-Coenzyme A acetyltransferase 2 (acetoacetyl Coenzyme A thiolase)	1.151 (0.695 to 1.98)	0.689
acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	0.937 (0.72 to 1.243)	0.599
acetyl-Coenzyme A carboxylase beta	1.556 (0.928 to 3.013)	0.331
acetylserotonin O-methyltransferase	1.304	no replicates
acetylserotonin O-methyltransferase-like	1.036 (0.955 to 1.124)	0.74
achaete-scute complex-like 2 (Drosophila)	0.837 (0.822 to 0.852)	0.065
acid phosphatase 1, soluble	0.551 (0.356 to 0.853)	0.403
acid phosphatase 2, lysosomal	0.824 (0.641 to 1.058)	0.581
acid phosphatase 5, tartrate resistant	0.619 (0.547 to 0.7)	0.16
acid phosphatase, prostate	0.912 (0.642 to 1.274)	0.557
acidic epididymal glycoprotein-like 1	1.464 (0.992 to 2.161)	0.506
aconitase 2, mitochondrial	1.024 (0.875 to 1.199)	0.903
acrosomal vesicle protein 1	0.914 (0.473 to 2.256)	0.803
actin binding LIM protein 1	1 (0.84 to 1.272)	0.997
actin related protein 2/3 complex, subunit 1A, 41kDa	1.037 (0.78 to 1.307)	0.803
actin related protein 2/3 complex, subunit 1B, 41kDa	0.864 (0.562 to 1.327)	0.791
actin related protein 2/3 complex, subunit 2, 34kDa	0.881 (0.558 to 1.285)	0.655
actin related protein 2/3 complex, subunit 3, 21kDa	0.67 (0.424 to 1.058)	0.542
actin related protein 2/3 complex, subunit 4, 20kDa	0.633 (0.458 to 0.957)	0.17
actin related protein 2/3 complex, subunit 5, 16kDa	1.337 (1.002 to 1.784)	0.498
actin, alpha 1, skeletal muscle	1.767	no replicates
actin, alpha 2, smooth muscle, aorta	2.389 (0.839 to 6.808)	0.558
actin, alpha, cardiac muscle	2.322 (1.78 to 3.028)	0.194
actin, beta	1.096 (0.88 to 1.843)	0.526
actin, gamma 1	0.928 (0.437 to 2.048)	0.829
actin, gamma 2, smooth muscle, enteric	0.697 (0.326 to 1.987)	0.424
actinin, alpha 1	0.559 (0.418 to 0.748)	0.296
actinin, alpha 2	1.214 (1.122 to 1.314)	0.246
actinin, alpha 3	0.816	no replicates
actinin, alpha 4	0.872 (0.74 to 1.171)	0.451
actin-like 7A	0.864 (0.727 to 1.026)	0.551
activated leukocyte cell adhesion molecule	1.348	no replicates
activating transcription factor 1	0.974 (0.555 to 1.37)	0.879
activating transcription factor 3	1.362 (0.329 to 7.281)	0.765
activating transcription factor 7	0.849 (0.635 to 1.011)	0.377
activator of S phase kinase	0.74	no replicates
activin A receptor type II-like 1	0.702 (0.219 to 1.466)	0.454
activin A receptor, type I	1.193 (1.123 to 1.268)	0.21
activin A receptor, type IB	0.878	no replicates
activin A receptor, type II	0.932 (0.384 to 1.714)	0.891
activin A receptor, type IIB	1.578 (1.172 to 2.124)	0.368
acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain	1.274 (0.961 to 1.69)	0.548
acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain	0.95 (0.729 to 1.237)	0.878
acyl-Coenzyme A dehydrogenase, long chain	0.91 (0.805 to 1.028)	0.581
acyl-Coenzyme A dehydrogenase, short/branched chain	0.887 (0.828 to 0.949)	0.328
acyl-Coenzyme A dehydrogenase, very long chain	1.047 (0.995 to 1.101)	0.533
acyl-Coenzyme A oxidase 1, palmitoyl	0.846 (0.578 to 1.237)	0.736
acyl-Coenzyme A oxidase 2, branched chain	1.574 (1.338 to 1.852)	0.219
acyl-Coenzyme A oxidase 3, pristanoyl	1.022 (0.83 to 1.551)	0.852
acyloxyacyl hydrolase (neutrophil)	0.863	no replicates
acylphosphatase 2, muscle type	0.755	no replicates
adaptor-related protein complex 1, beta 1 subunit	1.404 (0.973 to 1.855)	0.218
adaptor-related protein complex 1, gamma 1 subunit	0.872 (0.702 to 1.085)	0.644
adaptor-related protein complex 1, sigma 1 subunit	0.721 (0.46 to 0.922)	0.135
adaptor-related protein complex 2, beta 1 subunit	0.8 (0.64 to 1)	0.5
adaptor-related protein complex 2, mu 1 subunit	1.247 (1.193 to 1.303)	0.126
adaptor-related protein complex 3, beta 1 subunit	0.965 (0.8 to 1.165)	0.882
adaptor-related protein complex 3, beta 2 subunit	1.168 (0.867 to 1.771)	0.544
adaptor-related protein complex 3, delta 1 subunit	1.1 (0.994 to 1.216)	0.52
adaptor-related protein complex 3, sigma 1 subunit	1.063 (0.774 to 1.506)	0.686
adaptor-related protein complex 3, sigma 2 subunit	1.514 (1.389 to 1.65)	0.131
adaptor-related protein complex 4, beta 1 subunit	1.129 (0.687 to 1.856)	0.848

adducin 1 (alpha)	0.943 (0.751 to 1.185)	0.84
adenine phosphoribosyltransferase	1.34 (0.946 to 1.899)	0.555
adenomatous polyposis coli like	1.172 (1.04 to 1.321)	0.411
adenosine A2b receptor	0.786	no replicates
adenosine deaminase	0.938 (0.926 to 0.951)	0.131
adenosine deaminase, RNA-specific	0.895 (0.774 to 1.035)	0.586
adenosine deaminase, RNA-specific, B1 (RED1 homolog rat)	1.097 (0.586 to 1.494)	0.693
adenosine kinase	0.749 (0.581 to 1.017)	0.0406
adenosine monophosphate deaminase (isoform E)	0.825 (0.452 to 1.369)	0.612
adenosine monophosphate deaminase 1 (isoform M)	0.895	no replicates
adenosine monophosphate deaminase 2 (isoform L)	1.067 (0.857 to 1.328)	0.817
adenosylmethionine decarboxylase 1	1.941 (0.976 to 3.862)	0.511
adenylate cyclase 3	1	no replicates
adenylate cyclase 8 (brain)	1.012 (0.884 to 1.082)	0.806
adenylate cyclase 9	0.925 (0.674 to 1.426)	0.59
adenylate cyclase activating polypeptide 1 (pituitary)	1.107 (0.883 to 1.387)	0.73
adenylate cyclase activating polypeptide 1 (pituitary) receptor type I	0.69 (0.501 to 1.024)	0.219
adenylate kinase 1	1.414 (1.158 to 1.726)	0.333
adenylate kinase 3	0.773 (0.552 to 1.282)	0.423
adenylosuccinate lyase	0.67 (0.181 to 1.488)	0.605
adenylosuccinate synthase	0.954 (0.639 to 1.261)	0.765
adipose differentiation-related protein	0.868 (0.753 to 1.001)	0.501
adipose most abundant gene transcript 1	1.259 (1.016 to 1.56)	0.478
adipose specific 2	1.069 (1.045 to 1.093)	0.208
ADP-ribosylarginine hydrolase	0.733 (0.475 to 1.132)	0.605
ADP-ribosylation factor 3	0.902 (0.858 to 0.948)	0.288
ADP-ribosylation factor 4	1.445 (1.394 to 1.498)	0.0625
ADP-ribosylation factor 4-like	1.448	no replicates
ADP-ribosylation factor 5	0.935 (0.863 to 1.014)	0.562
ADP-ribosylation factor 6	0.431	no replicates
ADP-ribosylation factor domain protein 1, 64kDa	0.99 (0.744 to 1.316)	0.977
ADP-ribosylation factor guanine nucleotide-exchange factor 2 (brefeldin A-inhibited)	1.479 (1.424 to 1.537)	0.062
ADP-ribosylation factor related protein 1	0.884	no replicates
ADP-ribosylation factor-like 1	1.363 (0.846 to 2.939)	0.243
ADP-ribosylation factor-like 2	1.093 (1.053 to 1.135)	0.256
ADP-ribosylation factor-like 3	1.15 (0.96 to 1.377)	0.58
ADP-ribosylation factor-like 4	1.189 (1.169 to 1.209)	0.0616
ADP-ribosylation factor-like 7	1.068 (1.019 to 1.12)	0.395
ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)-like 1	0.661 (0.434 to 1.008)	0.506
ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase)-like 3	0.855 (0.774 to 0.945)	0.361
ADP-ribosyltransferase 1	0.884 (0.769 to 1.018)	0.542
ADP-ribosyltransferase 3	1.478 (0.791 to 2.762)	0.644
adrenergic, alpha-1A-, receptor	1.13 (0.835 to 1.53)	0.756
adrenergic, alpha-1B-, receptor	1.035 (0.914 to 1.172)	0.826
adrenergic, alpha-1D-, receptor	1.071 (0.928 to 1.252)	0.511
adrenergic, alpha-2B-, receptor	2.913 (2.347 to 3.616)	0.127
adrenergic, alpha-2C-, receptor	1.709 (0.978 to 2.984)	0.513
adrenergic, beta, receptor kinase 1	0.95 (0.866 to 1.042)	0.677
adrenergic, beta, receptor kinase 2	0.81 (0.553 to 1.162)	0.429
adrenergic, beta-1-, receptor	1.22 (1.194 to 1.246)	0.0683
adrenergic, beta-3-, receptor	0.912 (0.731 to 1.353)	0.572
adrenomedullin	0.922 (0.722 to 1.178)	0.797
adrenomedullin receptor	0.89 (0.542 to 1.153)	0.538
advillin	1.155 (0.453 to 2.214)	0.792
AE binding protein 1	1.285 (1.237 to 1.334)	0.0952
afamin	1.285 (0.815 to 2.026)	0.679
AFG3 ATPase family gene 3-like 2 (yeast)	0.58 (0.237 to 1.419)	0.652
aggrecan 1 (chondroitin sulfate proteoglycan 1, large aggregating proteoglycan, antigen identified by monoclonal antibody A0122)	0.243 (0.0797 to 0.738)	0.424
agouti related protein homolog (mouse)	0.85 (0.668 to 1)	0.318
agouti signaling protein, nonagouti homolog (mouse)	0.947 (0.689 to 1.313)	0.796
alanine-glyoxylate aminotransferase (oxalosis I; hyperoxaluria I; glycolicaciduria; serine-pyruvate aminotransferase)	0.496 (0.39 to 0.629)	0.209
alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)	0.959 (0.85 to 1.082)	0.788
albumin	0.793	no replicates
alcohol dehydrogenase 1A (class I), alpha polypeptide	0.775 (0.55 to 1.091)	0.592
alcohol dehydrogenase 1C (class I), gamma polypeptide	1	no replicates
alcohol dehydrogenase 4 (class II), pi polypeptide	0.757 (0.43 to 1.573)	0.544

alcohol dehydrogenase 5 (class III), chi polypeptide	1.077 (0.766 to 1.516)	0.863
alcohol dehydrogenase 6 (class V)	1.425	no replicates
alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide	1.053 (1.005 to 1.102)	0.465
alcohol dehydrogenase IB (class I), beta polypeptide	1.071 (0.792 to 1.317)	0.701
aldehyde dehydrogenase 1 family, member A1	1.614 (1.148 to 2.27)	0.394
aldehyde dehydrogenase 1 family, member A3	1.329 (0.996 to 1.841)	0.252
aldehyde dehydrogenase 1 family, member B1	0.949	no replicates
aldehyde dehydrogenase 2 family (mitochondrial)	1.403 (1.073 to 1.836)	0.427
aldehyde dehydrogenase 3 family, member A2	0.942 (0.787 to 1.103)	0.514
aldehyde dehydrogenase 3 family, member B1	0.737 (0.506 to 1.074)	0.567
aldehyde dehydrogenase 3 family, member B2	0.703 (0.562 to 0.88)	0.361
aldehyde dehydrogenase 3 family, memberA1	1.136 (0.83 to 1.696)	0.458
aldehyde dehydrogenase 4 family, member A1	1.346	no replicates
aldehyde dehydrogenase 5 family, member A1 (succinate-semialdehyde dehydrogenase)	0.708 (0.464 to 1.08)	0.563
aldehyde dehydrogenase 7 family, member A1	1.112 (0.855 to 1.448)	0.755
aldehyde dehydrogenase 9 family, member A1	0.964	no replicates
aldehyde oxidase 1	1.615	no replicates
aldo-keto reductase family 1, member A1 (aldehyde reductase)	1.207 (1.094 to 1.331)	0.306
aldo-keto reductase family 1, member B1 (aldose reductase)	1.16 (0.986 to 1.365)	0.529
aldo-keto reductase family 1, member B10 (aldose reductase)	1.183 (0.981 to 1.395)	0.0449
aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase)	1.196 (1.171 to 1.221)	0.0738
aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)	1.986 (1.027 to 3.839)	0.487
aldolase A, fructose-bisphosphate	0.742 (0.463 to 1.55)	0.508
aldolase B, fructose-bisphosphate	1.005 (0.832 to 1.279)	0.974
alkaline phosphatase, intestinal	2.183 (1.412 to 3.374)	0.324
alkaline phosphatase, liver/bone/kidney	1.068	no replicates
alkaline phosphatase, placental (Regan isozyme)	1.17 (0.743 to 2.087)	0.564
alkB, alkylation repair homolog (E. coli)	2.132 (1.982 to 2.292)	0.0608
alkylglycerone phosphate synthase	0.713 (0.557 to 0.914)	0.403
ALL1-fused gene from chromosome 1q	1.289	no replicates
allograft inflammatory factor 1	1.203 (0.773 to 1.872)	0.748
alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae)	0.96 (0.937 to 0.983)	0.331
alpha-1-microglobulin/bikunin precursor	1.082 (1.047 to 1.117)	0.25
alpha-2-glycoprotein 1, zinc	0.983 (0.762 to 1.477)	0.88
alpha-2-HS-glycoprotein	0.905 (0.69 to 1.186)	0.775
alpha-2-macroglobulin	1.29 (0.941 to 1.883)	0.108
amelogenin (X chromosome, amelogenesis imperfecta 1)	0.963 (0.714 to 1.299)	0.921
amelogenin (Y chromosome)	0.884 (0.756 to 1.101)	0.389
amiloride binding protein 1 (amine oxidase (copper-containing))	1.176 (0.54 to 2.424)	0.644
amiloride-sensitive cation channel 1, neuronal (degenerin)	1.13 (1.077 to 1.186)	0.239
amiloride-sensitive cation channel 2, neuronal	1.196 (1.008 to 1.419)	0.485
amine oxidase, copper containing 2 (retina-specific)	1.351 (0.559 to 3.269)	0.791
amine oxidase, copper containing 3 (vascular adhesion protein 1)	1.197 (0.812 to 1.765)	0.724
aminoacylase 1	1.219 (0.696 to 2.025)	0.454
aminolevulinate, delta-, dehydratase	1.095 (1.012 to 1.184)	0.455
aminolevulinate, delta-, synthase 1	1.453 (1.444 to 1.461)	0.0105
aminolevulinate, delta-, synthase 2 (sideroblastic/hypochromic anemia)	1.14 (0.912 to 1.425)	0.662
aminomethyltransferase (glycine cleavage system protein T)	1.046 (0.686 to 1.595)	0.932
amino-terminal enhancer of split	0.425	no replicates
amphiphysin (Stiff-Man syndrome with breast cancer 128kDa autoantigen)	1.147 (1.114 to 1.181)	0.133
amylase, alpha 1A; salivary	0.919	no replicates
amylo-1, 6-glucosidase, 4-alpha-glucanotransferase (glycogen debranching enzyme, glycogen storage disease type III)	0.66 (0.43 to 1.014)	0.51
amyloid beta (A4) precursor protein-binding, family A, member 2 (X11-like)	1.184 (1.06 to 1.41)	0.196
amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)	2.218 (2.154 to 2.284)	0.0234
amyloid beta (A4) precursor-like protein 1	1.047 (0.668 to 1.37)	0.725
amyloid beta (A4) precursor-like protein 2	0.872 (0.543 to 1.181)	0.626
amyloid beta precursor protein (cytoplasmic tail) binding protein 2	1.064 (1.033 to 1.097)	0.286
amyloid beta precursor protein binding protein 1, 59kDa	0.925 (0.267 to 2.929)	0.921
amyloid P component, serum	3.489 (1.248 to 9.752)	0.438
AND-1 protein	1.659 (1.598 to 1.721)	0.0465
angio-associated, migratory cell protein	1.121 (0.827 to 1.519)	0.771
angiogenin, ribonuclease, RNase A family, 5	0.828 (0.619 to 1.108)	0.635
angiopoietin 1	1.201 (0.875 to 1.649)	0.666
angiopoietin 2	1.899 (1.569 to 2.299)	0.184
angiopoietin-like 1	1.079 (0.863 to 1.265)	0.442

angiotensin II receptor, type 1	1.03 (0.946 to 1.152)	0.571
angiotensin II receptor, type 2	1.255 (0.936 to 1.684)	0.581
angiotensin II receptor-like 1	1.301 (1.262 to 1.341)	0.0734
angiotensin II receptor-like 2	1.108 (0.91 to 1.349)	0.694
angiotensinogen (serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 8)	0.654 (0.362 to 1.806)	0.493
ankyrin 1, erythrocytic	1.083 (1.062 to 1.105)	0.158
ankyrin 2, neuronal	1.116 (0.775 to 1.49)	0.627
ankyrin 3, node of Ranvier (ankyrin G)	1.407 (0.941 to 2.104)	0.552
annexin A1	0.612 (0.476 to 0.786)	0.3
annexin A10	1.197 (0.972 to 1.474)	0.547
annexin A11	0.826 (0.69 to 0.939)	0.174
annexin A13	1.117	no replicates
annexin A2	0.57 (0.132 to 1.156)	0.222
annexin A3	0.898 (0.869 to 0.927)	0.185
annexin A5	1.022 (0.579 to 1.804)	0.975
annexin A6	1.019 (0.795 to 1.264)	0.848
annexin A7	0.925 (0.78 to 0.991)	0.262
annexin A8	1.042 (0.871 to 1.245)	0.857
annexin A9	2.64 (1.035 to 6.733)	0.488
anterior gradient 2 homolog (Xenepus laevis)	0.83 (0.48 to 1.233)	0.322
antigen identified by monoclonal antibody MRC OX-2	1.065 (0.798 to 1.545)	0.68
anti-Mullerian hormone	0.459 (0.201 to 1.05)	0.519
APG12 autophagy 12-like (S. cerevisiae)	1.207 (1.07 to 1.394)	0.136
APG5 autophagy 5-like (S. cerevisiae)	1.35 (1.078 to 1.691)	0.41
apical protein-like (Xenopus laevis)	0.919 (0.779 to 1.085)	0.702
apolipoprotein A-I	0.913 (0.4 to 1.742)	0.751
apolipoprotein A-II	0.894 (0.761 to 1.05)	0.613
apolipoprotein B (including Ag(x) antigen)	1.39 (1.077 to 1.628)	0.125
apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1	1.416 (0.938 to 2.137)	0.554
apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 2	0.955 (0.652 to 1.348)	0.711
apolipoprotein C-I	1.32 (0.887 to 2.777)	0.226
apolipoprotein C-II	0.851 (0.649 to 1.232)	0.488
apolipoprotein C-III	1.398 (1.021 to 1.913)	0.48
apolipoprotein C-IV	1.911	no replicates
apolipoprotein D	0.984 (0.706 to 1.372)	0.97
apolipoprotein F	1.224 (0.873 to 1.737)	0.416
apolipoprotein H (beta-2-glycoprotein I)	1.284 (1.095 to 1.505)	0.36
apoptosis inhibitor 5	0.889 (0.787 to 1.005)	0.513
apoptosis-associated tyrosine kinase	3.011 (2.566 to 3.534)	0.0918
aquaporin 1 (channel-forming integral protein, 28kDa)	0.447 (0.213 to 0.936)	0.473
aquaporin 2 (collecting duct)	1.203	no replicates
aquaporin 3	0.882 (0.406 to 1.754)	0.622
aquaporin 5	1.5 (1.316 to 1.813)	0.0527
aquaporin 6, kidney specific	1.378 (1.02 to 1.862)	0.48
aquaporin 7	1.011 (0.211 to 3.08)	0.982
aquaporin 8	1.52 (1.4 to 1.649)	0.123
arachidonate 12-lipoxygenase	1.348 (1.075 to 1.988)	0.266
arachidonate 12-lipoxygenase, 12R type	1.659 (0.744 to 4.125)	0.416
arachidonate 15-lipoxygenase	0.937 (0.802 to 1.098)	0.325
arachidonate 15-lipoxygenase, second type	2.246 (0.858 to 9.008)	0.373
arachidonate 5-lipoxygenase	1.26 (1.049 to 1.514)	0.427
arachidonate 5-lipoxygenase-activating protein	1.171 (1 to 1.336)	0.203
archain 1	0.929 (0.833 to 1.036)	0.621
Arg/Abl-interacting protein ArgBP2	1.024 (0.911 to 1.084)	0.592
arginase, type II	1.147 (0.818 to 1.49)	0.354
arginine vasopressin (neurophysin II, antidiuretic hormone, diabetes insipidus, neurohypophyseal)	1.137 (0.727 to 1.549)	0.632
arginine vasopressin receptor 1A	1.061 (0.979 to 1.15)	0.594
arginine vasopressin receptor 1B	1.683 (1.152 to 2.459)	0.401
arginine vasopressin receptor 2 (nephrogenic diabetes insipidus)	0.476 (0.228 to 0.992)	0.497
arginine-rich, mutated in early stage tumors	0.974 (0.685 to 1.411)	0.911
argininosuccinate lyase	0.829 (0.462 to 1.186)	0.43
argininosuccinate synthetase	0.962 (0.893 to 1.036)	0.691
arginyl-tRNA synthetase	1.076 (0.686 to 1.372)	0.59
ariadne homolog 2 (Drosophila)	1.094	no replicates
aristaless-like homeobox 3	1.313 (0.849 to 2.316)	0.182
armadillo repeat gene deletes in velocardiofacial syndrome	0.82 (0.575 to 1.084)	0.185
ARP1 actin-related protein 1 homolog A, centractin alpha (yeast)	0.699 (0.356 to 1.372)	0.689
ARP2 actin-related protein 2 homolog (yeast)	0.9 (0.791 to 1.025)	0.566

ARP3 actin-related protein 3 homolog (yeast)	0.955 (0.791 to 1.153)	0.847
arrestin 3, retinal (X-arrestin)	1.013 (0.878 to 1.161)	0.84
arrestin, beta 1	1.253 (0.967 to 1.623)	0.544
arsA arsenite transporter, ATP-binding, homolog 1 (bacterial)	0.788	no replicates
artemin	1.249 (0.771 to 1.831)	0.475
aryl hydrocarbon receptor	0.766 (0.54 to 1.088)	0.586
aryl hydrocarbon receptor interacting protein	0.613 (0.381 to 0.988)	0.492
aryl hydrocarbon receptor nuclear translocator	0.793 (0.71 to 0.911)	0.0869
aryl hydrocarbon receptor nuclear translocator-like	2.065 (1.268 to 3.363)	0.377
arylacetamide deacetylase (esterase)	1.107 (0.818 to 1.402)	0.588
arylsulfatase A	0.959 (0.766 to 1.209)	0.638
arylsulfatase D	0.837	no replicates
arylsulfatase E (chondrodysplasia punctata 1)	1.158 (1.028 to 1.461)	0.334
arylsulfatase F	1.147 (1.004 to 1.309)	0.49
ash2 (absent, small, or homeotic)-like (Drosophila)	0.749 (0.613 to 0.904)	0.0368
asialoglycoprotein receptor 1	1.366	no replicates
asialoglycoprotein receptor 2	1.057 (0.735 to 1.571)	0.747
asparagine synthetase	1.098 (0.81 to 1.515)	0.606
asparaginyl-tRNA synthetase	1.162 (0.846 to 1.7)	0.375
aspartate beta-hydroxylase	0.443	no replicates
aspartoacylase (aminoacylase 2, Canavan disease)	1.113	no replicates
aspartylglucosaminidase	0.982 (0.798 to 1.209)	0.945
aspartyl-tRNA synthetase	0.942	no replicates
associated molecule with the SH3 domain of STAM	1.702 (1.106 to 2.621)	0.434
ataxia telangiectasia and Rad3 related	1.49 (1.172 to 1.895)	0.345
ataxin 2 related protein	1.413 (1.165 to 1.714)	0.324
atonal homolog 1 (Drosophila)	0.847 (0.768 to 0.934)	0.339
ATP binding protein associated with cell differentiation	1.063 (1.034 to 1.093)	0.271
ATP citrate lyase	0.743 (0.545 to 0.884)	0.195
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1	1.596 (1.534 to 1.66)	0.0539
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9) isoform 3	0.577 (0.307 to 1.086)	0.544
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 1	1.069 (1.05 to 1.088)	0.168
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	1.118 (1.072 to 1.166)	0.229
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	1.259 (0.782 to 2.279)	0.393
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit e	0.674 (0.236 to 1.273)	0.534
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f, isoform 2	1.638 (1.151 to 2.331)	0.395
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F6	1.032 (0.783 to 1.454)	0.828
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit g	0.691 (0.259 to 2.062)	0.452
ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, cardiac muscle	1.053 (0.827 to 1.341)	0.867
ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide	0.926 (0.923 to 0.929)	0.0275
ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit	0.421 (0.125 to 1.414)	0.605
ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	1.732 (1.563 to 1.92)	0.118
ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (oligomycin sensitivity conferring protein)	2.876 (1.289 to 6.418)	0.414
ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	1.344 (1.182 to 1.527)	0.261
ATPase, Ca++ transporting, plasma membrane 1	0.74 (0.451 to 1.214)	0.652
ATPase, Ca++ transporting, plasma membrane 4	1.746 (1.065 to 4.68)	0.376
ATPase, Ca++ transporting, ubiquitous	1.216 (0.472 to 3.131)	0.87
ATPase, Class I, type 8B, member 1	1.06 (0.973 to 1.153)	0.619
ATPase, Cu++ transporting, alpha polypeptide (Menkes syndrome)	0.655	no replicates
ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 1	1.855 (0.973 to 3.534)	0.514
ATPase, H+ transporting, lysosomal 14kDa, V1 subunit F	1.655 (0.682 to 3.62)	0.408
ATPase, H+ transporting, lysosomal 16kDa, V0 subunit c	1.205 (1.168 to 1.244)	0.107
ATPase, H+ transporting, lysosomal 21kDa, V0 subunit c"	1.414 (1.25 to 1.997)	0.0573
ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E isoform 1	0.749	no replicates
ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C, isoform 1	0.889 (0.736 to 1.074)	0.647
ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B, isoform 1 (Renal tubular acidosis with deafness)	0.952 (0.737 to 1.424)	0.687
ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B, isoform 2	2.48 (1.518 to 4.051)	0.315
ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A	1.47 (1.116 to 2.178)	0.0364
ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e	0.737	no replicates
ATPase, H+ transporting, lysosomal interacting protein 1	0.828 (0.709 to 1.115)	0.0737
ATPase, H+ transporting, lysosomal interacting protein 2	1.258 (0.857 to 2.119)	0.221

ATPase, H+ transporting, lysosomal V0 subunit a isoform 1	1.241 (0.97 to 1.965)	0.148
ATPase, H+/K+ exchanging, alpha polypeptide	0.953 (0.524 to 1.234)	0.828
ATPase, H+/K+ exchanging, beta polypeptide	1.137 (1.015 to 1.273)	0.462
ATPase, H+/K+ transporting, nongastric, alpha polypeptide	1.315 (0.413 to 1.986)	0.53
ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide	0.971 (0.967 to 0.975)	0.0872
ATPase, Na+/K+ transporting, alpha 3 polypeptide	1.63	no replicates
ATPase, Na+/K+ transporting, beta 1 polypeptide	0.624 (0.313 to 1.243)	0.618
ATPase, Na+/K+ transporting, beta 2 polypeptide	0.608 (0.392 to 0.943)	0.46
ATP-binding cassette, sub-family A (ABC1), member 1	0.977 (0.837 to 1.141)	0.905
ATP-binding cassette, sub-family A (ABC1), member 3	1.495 (1.143 to 2.348)	0.218
ATP-binding cassette, sub-family A (ABC1), member 4	1.188 (0.82 to 3.515)	0.563
ATP-binding cassette, sub-family A (ABC1), member 8	1.166 (1.137 to 1.197)	0.105
ATP-binding cassette, sub-family B (MDR/TAP), member 1	1.274 (0.559 to 3.111)	0.547
ATP-binding cassette, sub-family B (MDR/TAP), member 11	1.037 (0.949 to 1.134)	0.75
ATP-binding cassette, sub-family B (MDR/TAP), member 4	0.834 (0.795 to 0.874)	0.163
ATP-binding cassette, sub-family B (MDR/TAP), member 7	1.185 (0.954 to 1.67)	0.43
ATP-binding cassette, sub-family C (CFTR/MRP), member 1	1.084 (0.654 to 1.793)	0.807
ATP-binding cassette, sub-family C (CFTR/MRP), member 3	0.753 (0.611 to 0.864)	0.116
ATP-binding cassette, sub-family C (CFTR/MRP), member 6	1.409 (1.289 to 1.54)	0.162
ATP-binding cassette, sub-family C (CFTR/MRP), member 8	0.908 (0.562 to 1.426)	0.659
ATP-binding cassette, sub-family D (ALD), member 3	0.816 (0.629 to 0.927)	0.105
ATP-binding cassette, sub-family D (ALD), member 4	1.154 (0.633 to 2.104)	0.851
ATP-binding cassette, sub-family E (OABP), member 1	0.986 (0.949 to 1.023)	0.763
ATP-binding cassette, sub-family F (GCN20), member 2	1.084 (0.961 to 1.222)	0.625
ATP-binding cassette, sub-family G (WHITE), member 2	0.945	no replicates
ATX1 antioxidant protein 1 homolog (yeast)	1.585	no replicates
AU RNA binding protein/enoyl-Coenzyme A hydratase	1.577 (0.849 to 2.929)	0.596
autoimmune regulator (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy)	1.153 (0.617 to 1.791)	0.701
avian lymphoma virus-derived transforming sequence (BLYM)	1.341 (1.078 to 1.668)	0.407
axin 2 (conductin, axil)	0.923 (0.89 to 0.958)	0.273
axonal transport of synaptic vesicles	1.656 (1.158 to 2.369)	0.393
B lymphocyte gene 1	0.96 (0.542 to 2.11)	0.929
B lymphoid tyrosine kinase	0.973 (0.959 to 0.986)	0.294
B lymphoma Mo-MLV insertion region (mouse)	1.161 (1.043 to 1.292)	0.397
B melanoma antigen	0.915 (0.777 to 1.077)	0.683
B7 gene	0.929 (0.586 to 1.726)	0.767
bactericidal/permeability-increasing protein	1.3 (0.705 to 2.986)	0.433
baculoviral IAP repeat-containing 1	1.032 (0.84 to 1.154)	0.789
baculoviral IAP repeat-containing 2	1.589 (0.759 to 7.435)	0.44
baculoviral IAP repeat-containing 3	1.215 (0.907 to 1.711)	0.234
baculoviral IAP repeat-containing 4	0.846 (0.556 to 1.582)	0.506
baculoviral IAP repeat-containing 5 (survivin)	1.126 (0.816 to 1.77)	0.661
BAF53	0.601 (0.181 to 1.243)	0.488
bagpipe homeobox homolog 1 (Drosophila)	1.756	no replicates
BAI1-associated protein 2	0.74 (0.311 to 1.431)	0.441
BAI1-associated protein 3	0.804 (0.429 to 1.617)	0.362
BarH-like homeobox 2	1.409 (1.268 to 1.566)	0.19
barrier to autointegration factor 1	1.027 (0.99 to 1.065)	0.6
basic helix-loop-helix domain containing, class B, 2	1.153 (0.969 to 1.372)	0.564
basic leucine zipper nuclear factor 1 (JEM-1)	1.089 (1.073 to 1.105)	0.108
basic leucine zipper transcription factor, ATF-like	1.084 (1.066 to 1.101)	0.126
basic transcription element binding protein 1	0.944 (0.556 to 1.834)	0.886
basic transcription factor 3	1.089 (0.846 to 1.629)	0.716
basic transcription factor 3, like 1	0.836 (0.676 to 1.034)	0.555
basic transcription factor 3, like 2	0.844 (0.605 to 1.208)	0.484
basonuclin	0.93 (0.665 to 1.326)	0.752
B-cell CLL/lymphoma 10	1.182 (0.578 to 3.615)	0.619
B-cell CLL/lymphoma 6 (zinc finger protein 51)	1.153 (0.663 to 2.113)	0.534
B-cell CLL/lymphoma 7B	1.63 (1.323 to 2.007)	0.256
B-cell CLL/lymphoma 7C	1.089 (0.827 to 1.435)	0.809
B-cell CLL/lymphoma 9	0.881 (0.735 to 1.057)	0.613
B-cell growth factor 1 (12kD)	0.96 (0.901 to 1.022)	0.633
B-cell translocation gene 1, anti-proliferative	1.804 (0.746 to 6.884)	0.478
BCL2/adenovirus E1B 19kDa interacting protein 3	1.13 (0.731 to 1.747)	0.826
BCL2/adenovirus E1B 19kDa interacting protein 3-like	1.098 (0.725 to 1.681)	0.647
BCL2-antagonist/killer 1	0.784 (0.697 to 0.881)	0.285
BCL2-associated athanogene 2	0.884 (0.706 to 1.109)	0.683
BCL2-like 11 (apoptosis facilitator)	1.442 (1.115 to 1.864)	0.39
BCS1-like (yeast)	0.52 (0.312 to 0.866)	0.422

beaded filament structural protein 1, filensin	1.211 (0.744 to 2.524)	0.66
beaded filament structural protein 2, phakinin	3.146 (0.524 to 18.9)	0.638
beclin 1 (coiled-coil, myosin-like BCL2 interacting protein)	0.994 (0.862 to 1.147)	0.975
benzodiazapine receptor (peripheral)	0.617 (0.361 to 1.143)	0.133
BET1 homolog (S. cerevisiae)	0.845 (0.792 to 0.902)	0.233
beta-2-microglobulin	0.731 (0.582 to 0.917)	0.399
betacellulin	0.845 (0.765 to 0.934)	0.34
betaine-homocysteine methyltransferase	0.651 (0.56 to 0.758)	0.217
B-factor, properdin	0.885 (0.821 to 0.954)	0.35
BH3 interacting domain death agonist	1.204 (0.959 to 1.681)	0.254
BH-protocadherin (brain-heart)	1.171 (1.136 to 1.208)	0.123
Bicaudal D homolog 1 (Drosophila)	1.5 (1.198 to 1.88)	0.323
biglycan	0.999 (0.818 to 1.218)	0.995
bile acid Coenzyme A: amino acid N-acyltransferase (glycine N-choloyltransferase)	1.247 (0.514 to 2.69)	0.692
biliverdin reductase A	1.154 (1.013 to 1.315)	0.47
biliverdin reductase B (flavin reductase (NADPH))	0.968 (0.542 to 1.678)	0.9
biotinidase	1.099 (0.525 to 3.054)	0.818
bladder cancer associated protein	1.195 (0.941 to 1.519)	0.592
bleomycin hydrolase	2.194 (1.229 to 6.395)	0.28
Bloom syndrome	1.068 (0.718 to 1.46)	0.712
BMX non-receptor tyrosine kinase	0.886 (0.621 to 1.125)	0.57
bombesin-like receptor 3	1.463 (1.024 to 2.764)	0.355
bone gamma-carboxyglutamate (gla) protein (osteocalcin)	1.084	no replicates
bone marrow stromal cell antigen 1	1.029 (0.808 to 1.32)	0.797
bone marrow stromal cell antigen 2	1.29 (1.239 to 1.352)	0.00975
bone morphogenetic protein 1	1.029 (0.861 to 1.23)	0.899
bone morphogenetic protein 15	1.427 (1.207 to 1.779)	0.0905
bone morphogenetic protein 3 (osteogenic)	1.492 (1.291 to 1.724)	0.221
bone morphogenetic protein 4	1.164 (0.985 to 1.375)	0.531
bone morphogenetic protein 6	1.124 (0.969 to 1.304)	0.576
bone morphogenetic protein 7 (osteogenic protein 1)	1.339 (1.254 to 1.43)	0.141
bone morphogenetic protein receptor, type IA	1.019 (0.703 to 1.477)	0.968
bone morphogenetic protein receptor, type IB	1.217 (0.81 to 1.83)	0.714
bone morphogenetic protein receptor, type II (serine/threonine kinase)	1.409 (0.824 to 2.411)	0.638
bradykinin receptor B1	0.804 (0.629 to 1.028)	0.537
bradykinin receptor B2	1.107 (1.09 to 1.125)	0.0984
brain-specific angiogenesis inhibitor 1	0.919 (0.774 to 1.064)	0.459
brain-specific angiogenesis inhibitor 2	0.85 (0.583 to 1.238)	0.74
brain-specific angiogenesis inhibitor 3	0.987 (0.964 to 1.012)	0.692
branched chain alpha-ketoacid dehydrogenase kinase	1.414 (1.162 to 1.72)	0.328
branched chain aminotransferase 1, cytosolic	1.065 (0.858 to 1.321)	0.819
branched chain aminotransferase 2, mitochondrial	0.983 (0.716 to 1.212)	0.923
branched chain keto acid dehydrogenase E1, beta polypeptide (maple syrup urine disease)	1.024 (1.011 to 1.038)	0.324
BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase)	1.316 (1.306 to 1.327)	0.0185
breakpoint cluster region	1.046 (0.84 to 1.577)	0.771
breast cancer 1, early onset	0.909 (0.61 to 1.071)	0.403
breast cancer anti-estrogen resistance 3	0.713 (0.389 to 1.063)	0.387
breast carcinoma amplified sequence 1	0.734 (0.568 to 0.948)	0.44
brefeldin A-inhibited guanine nucleotide-exchange protein 1	1.088 (0.935 to 1.364)	0.541
BRF1 homolog, subunit of RNA polymerase III transcription initiation factor IIIB (S. cerevisiae)	1.417 (0.981 to 2.309)	0.0898
bridging integrator 1	1.112 (0.523 to 3.566)	0.874
bromodomain and PHD finger containing, 1	2.012 (0.924 to 7.622)	0.406
bromodomain containing 2	1.743 (0.751 to 4.043)	0.629
bromodomain, testis-specific	0.961 (0.865 to 1.068)	0.771
Bruton agammaglobulinemia tyrosine kinase	1.077 (0.939 to 1.235)	0.685
BTB and CNC homology 1, basic leucine zipper transcription factor 1	1.065 (0.732 to 1.484)	0.787
BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.185 (0.777 to 1.695)	0.371
BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	0.714 (0.396 to 1.288)	0.67
bullous pemphigoid antigen 1, 230/240kDa	1.066 (0.755 to 1.928)	0.788
Burkitt lymphoma receptor 1, GTP binding protein (chemokine (C-X-C motif) receptor 5)	1.639	no replicates
butyrobetaine (gamma), 2-oxoglutarate dioxygenase (gamma-butyrobetaine hydroxylase) 1	1.16 (0.974 to 1.382)	0.552
butyrophilin, subfamily 1, member A1	0.935 (0.922 to 0.947)	0.126
butyrylcholinesterase	0.845 (0.585 to 1.045)	0.459
Ca2+-dependent activator protein for secretion	0.674 (0.271 to 1.076)	0.478
cadherin 10, type 2 (T2-cadherin)	0.715 (0.355 to 1.19)	0.453

cadherin 11, type 2, OB-cadherin (osteoblast)	1.341	no replicates
cadherin 15, M-cadherin (myotubule)	1.113 (1.108 to 1.119)	0.0296
cadherin 16, KSP-cadherin	1.022 (0.977 to 1.068)	0.715
cadherin 18, type 2	0.911 (0.793 to 0.981)	0.313
calbindin 1, 28kDa	0.801 (0.438 to 1.838)	0.656
calbindin 2, 29kDa (calretinin)	1.283 (0.764 to 1.933)	0.312
calbindin 3, (vitamin D-dependent calcium binding protein)	1.335 (0.712 to 4.344)	0.404
calcitonin/calcitonin-related polypeptide, alpha	1.336 (1.278 to 1.397)	0.0971
calcium channel, voltage-dependent, alpha 1E subunit	1.312 (0.954 to 1.803)	0.551
calcium channel, voltage-dependent, alpha 1F subunit	0.792 (0.3 to 2.004)	0.599
calcium channel, voltage-dependent, alpha 2/delta subunit 1	2.142 (1.149 to 3.991)	0.436
calcium channel, voltage-dependent, beta 1 subunit	0.807 (0.456 to 1.474)	0.543
calcium channel, voltage-dependent, beta 3 subunit	1.152 (0.929 to 1.429)	0.629
calcium channel, voltage-dependent, beta 4 subunit	0.9 (0.615 to 1.238)	0.516
calcium channel, voltage-dependent, gamma subunit 1	1.128 (1.11 to 1.146)	0.0828
calcium channel, voltage-dependent, L type, alpha 1C subunit	1.351 (1.343 to 1.358)	0.0112
calcium channel, voltage-dependent, L type, alpha 1D subunit	0.675 (0.41 to 1.11)	0.574
calcium channel, voltage-dependent, L type, alpha 1S subunit	2.231 (0.811 to 6.137)	0.573
calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	1.615 (1.007 to 2.785)	0.247
calcium modulating ligand	1.325 (1.094 to 1.713)	0.0573
calcium/calmodulin-dependent protein kinase I	1.297 (0.87 to 1.885)	0.214
calcium/calmodulin-dependent serine protein kinase (MAGUK family)	1.181 (1.058 to 1.319)	0.373
calcium-sensing receptor (hypocalciuric hypercalcemia 1, severe neonatal hyperparathyroidism)	1.124 (0.853 to 2.682)	0.62
calcyphosine	1.101 (0.947 to 1.24)	0.35
caldesmon 1	1.144 (0.869 to 1.512)	0.393
calmodulin 1 (phosphorylase kinase, delta)	1.145 (0.681 to 2.023)	0.563
calmodulin 2 (phosphorylase kinase, delta)	1.221 (1.167 to 1.296)	0.0233
calmodulin 3 (phosphorylase kinase, delta)	1.032 (0.625 to 1.759)	0.926
calpain 3, (p94)	0.924 (0.634 to 1.291)	0.737
calpain 5	1.306 (1.054 to 1.618)	0.431
calponin 1, basic, smooth muscle	1.036 (0.744 to 1.262)	0.852
calponin 2	1.195 (1.179 to 1.211)	0.047
calponin 3, acidic	0.972 (0.686 to 1.311)	0.852
calreticulin	1.181 (0.75 to 2.162)	0.507
calsequestrin 1 (fast-twitch, skeletal muscle)	0.976 (0.506 to 1.622)	0.951
calsequestrin 2 (cardiac muscle)	1.138 (1.034 to 1.252)	0.407
calumenin	1.177 (0.974 to 1.423)	0.547
cAMP responsive element binding protein 1	0.897 (0.723 to 1.05)	0.432
cAMP responsive element binding protein-like 1	1.049 (0.706 to 1.617)	0.771
cAMP responsive element binding protein-like 2	0.678 (0.367 to 1.253)	0.641
cancer/testis antigen 1	0.995 (0.432 to 1.573)	0.985
cannabinoid receptor 1 (brain)	0.668 (0.589 to 0.758)	0.193
cannabinoid receptor 2 (macrophage)	0.958 (0.854 to 1.18)	0.721
capping protein (actin filament) muscle Z-line, alpha 1	1.548 (0.905 to 3.155)	0.202
capping protein (actin filament) muscle Z-line, beta	1.324 (1.006 to 1.592)	0.183
capping protein (actin filament), gelsolin-like	1.111 (0.754 to 1.334)	0.383
carbamoyl-phosphate synthetase 1, mitochondrial	2.456 (1.582 to 3.3)	0.057
carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase	0.968 (0.839 to 1.075)	0.576
carbohydrate (keratan sulfate Gal-6) sulfotransferase 1	1.187 (1.063 to 1.325)	0.364
carbonic anhydrase III, muscle specific	0.796 (0.541 to 1.171)	0.66
carbonic anhydrase IV	1.146 (0.588 to 2.013)	0.525
carbonic anhydrase VA, mitochondrial	1.13 (0.807 to 1.583)	0.778
carbonic anhydrase VI	1.237 (1.067 to 1.433)	0.386
carbonic anhydrase VII	1.123 (0.58 to 1.828)	0.572
carbonic anhydrase VIII	0.552 (0.14 to 1.388)	0.485
carbonic anhydrase XI	0.989 (0.836 to 1.171)	0.96
carbonic anhydrase XII	0.954 (0.71 to 1.281)	0.898
carbonyl reductase 1	0.738 (0.215 to 2.178)	0.465
carboxyl ester lipase (bile salt-stimulated lipase)	0.807 (0.662 to 0.962)	0.0717
carboxyl ester lipase pseudogene	0.839 (0.371 to 1.511)	0.615
carboxylesterase 1 (monocyte/macrophage serine esterase 1)	0.884 (0.793 to 0.985)	0.457
carboxypeptidase A1 (pancreatic)	0.755 (0.529 to 1.399)	0.302
carboxypeptidase A2 (pancreatic)	1.517 (1.063 to 2.165)	0.45
carboxypeptidase A3 (mast cell)	0.482 (0.1 to 2.151)	0.497
carboxypeptidase B1 (tissue)	1.218 (0.825 to 2.182)	0.287
carboxypeptidase B2 (plasma, carboxypeptidase U)	1.624 (1.255 to 2.101)	0.311
carboxypeptidase M	1.506 (1.102 to 2.058)	0.415
carboxypeptidase N, polypeptide 1, 50kD	0.928 (0.902 to 0.955)	0.232

carboxypeptidase Z	0.992 (0.942 to 1.043)	0.896
carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)	0.875	no replicates
carcinoembryonic antigen-related cell adhesion molecule 3	1.369 (1.023 to 2.478)	0.224
carcinoembryonic antigen-related cell adhesion molecule 4	1.314 (0.991 to 1.692)	0.22
carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)	0.756 (0.122 to 1.862)	0.593
carcinoembryonic antigen-related cell adhesion molecule 7	1.047 (0.63 to 1.47)	0.815
carcinoembryonic antigen-related cell adhesion molecule 8	1.488 (1.085 to 1.756)	0.128
cardiotrophin 1	0.919 (0.761 to 1.039)	0.469
carnitine acetyltransferase	0.817 (0.562 to 1.219)	0.177
carnitine palmitoyltransferase 1A (liver)	0.939 (0.77 to 1.236)	0.699
carnitine palmitoyltransferase 1B (muscle)	0.992 (0.877 to 1.311)	0.936
carnitine palmitoyltransferase II	1.22 (1.14 to 1.295)	0.0331
cartilage intermediate layer protein, nucleotide pyrophosphohydrolase	1.348 (0.99 to 2.895)	0.21
cartilage linking protein 1	1.091 (0.959 to 1.242)	0.622
Cas-Br-M (murine) ecotropic retroviral transforming sequence b	1.014 (0.813 to 1.118)	0.866
casein alpha s1	1.178 (0.536 to 3.206)	0.641
casein beta	0.925 (0.706 to 1.12)	0.515
casein kappa	1.125 (0.998 to 1.505)	0.313
casein kinase 1, alpha 1	0.371	no replicates
casein kinase 1, delta	1.568 (0.865 to 3.658)	0.41
casein kinase 1, epsilon	0.634 (0.507 to 0.794)	0.292
casein kinase 1, gamma 2	0.748 (0.397 to 1.408)	0.726
casein kinase 1, gamma 3	1.033 (0.903 to 1.243)	0.768
casein kinase 2, alpha prime polypeptide	0.769 (0.458 to 1.047)	0.239
caspase 10, apoptosis-related cysteine protease	0.957 (0.726 to 1.262)	0.9
caspase 13, apoptosis-related cysteine protease	0.9 (0.585 to 1.358)	0.707
caspase 5, apoptosis-related cysteine protease	1.306 (1.026 to 1.887)	0.29
caspase 8, apoptosis-related cysteine protease	0.762 (0.639 to 0.908)	0.364
caspase 9, apoptosis-related cysteine protease	1.063 (0.781 to 1.447)	0.875
catalase	1.074 (0.998 to 1.155)	0.508
catenin (cadherin-associated protein), alpha-like 1	0.946 (0.658 to 1.36)	0.904
cathelicidin antimicrobial peptide	0.821 (0.556 to 1.697)	0.491
cathepsin B	1.33 (1.266 to 1.397)	0.108
cathepsin E	0.982 (0.861 to 1.09)	0.742
cathepsin F	0.953 (0.852 to 1.058)	0.519
cathepsin G	1.003 (0.871 to 1.156)	0.986
cathepsin K (pycnodysostosis)	0.772 (0.434 to 1.069)	0.464
cathepsin L	1.086	no replicates
cathepsin L2	1.061 (0.985 to 1.143)	0.572
cathepsin O	1.024 (0.881 to 1.141)	0.786
cathepsin S	1.347 (0.809 to 3.468)	0.44
cathepsin Z	1.286 (1.118 to 1.687)	0.204
caudal type homeo box transcription factor 1	1.222 (0.669 to 2.163)	0.4
caudal type homeo box transcription factor 2	1.233 (1.004 to 1.457)	0.195
caudal type homeo box transcription factor 4	0.889 (0.697 to 1.097)	0.236
caveolin 1, caveolae protein, 22kDa	1.05 (0.841 to 1.31)	0.863
caveolin 3	0.952 (0.941 to 0.963)	0.142
Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	0.626 (0.336 to 1.033)	0.292
CCAAT/enhancer binding protein (C/EBP), beta	0.612 (0.213 to 1.147)	0.271
CCAAT/enhancer binding protein (C/EBP), delta	0.737 (0.196 to 1.995)	0.701
CCAAT/enhancer binding protein (C/EBP), epsilon	0.729 (0.435 to 1.115)	0.203
CCAAT/enhancer binding protein (C/EBP), gamma	1.223 (1.133 to 1.336)	0.0524
CD14 antigen	0.984 (0.504 to 2.262)	0.974
CD151 antigen	1.272 (0.963 to 1.62)	0.127
CD1B antigen, b polypeptide	1.071 (0.936 to 1.526)	0.605
CD1D antigen, d polypeptide	1.104 (1.016 to 1.199)	0.445
CD2 antigen (p50), sheep red blood cell receptor	1.567 (0.757 to 4.619)	0.213
CD22 antigen	1.002 (0.892 to 1.127)	0.987
CD28 antigen (Tp44)	1.132 (0.815 to 1.573)	0.77
CD37 antigen	1.553 (1.303 to 1.849)	0.241
CD38 antigen (p45)	0.846 (0.738 to 1.028)	0.114
CD3D antigen, delta polypeptide (TiT3 complex)	1.001 (0.93 to 1.078)	0.989
CD3E antigen, epsilon polypeptide (TiT3 complex)	1.448 (0.981 to 2.031)	0.139
CD3G antigen, gamma polypeptide (TiT3 complex)	1.547 (1.54 to 1.554)	0.00676
CD3Z antigen, zeta polypeptide (TiT3 complex)	1.734 (1.498 to 2.006)	0.165
CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	1.101 (0.691 to 3.53)	0.82
CD48 antigen (B-cell membrane protein)	1.479 (1.003 to 2.206)	0.131

CD5 antigen-like (scavenger receptor cysteine rich family)	1.034 (0.795 to 1.205)	0.738
CD58 antigen, (lymphocyte function-associated antigen 3)	1.049 (0.82 to 1.288)	0.65
CD6 antigen	1.331 (1.092 to 1.738)	0.175
CD63 antigen (melanoma 1 antigen)	1.19 (0.764 to 1.958)	0.589
CD68 antigen	0.979	no replicates
CD69 antigen (p60, early T-cell activation antigen)	1.03 (0.713 to 1.418)	0.896
CD7 antigen (p41)	0.839 (0.546 to 1.451)	0.604
CD72 antigen	1.053 (1.029 to 1.078)	0.268
CD79A antigen (immunoglobulin-associated alpha)	1.265 (0.745 to 2.363)	0.496
CD8 antigen, alpha polypeptide (p32)	1.359 (0.906 to 2.037)	0.588
CD80 antigen (CD28 antigen ligand 1, B7-1 antigen)	1.117 (0.78 to 2.031)	0.749
CD81 antigen (target of antiproliferative antibody 1)	1.065 (0.673 to 1.508)	0.703
CD97 antigen	0.937 (0.609 to 1.79)	0.861
CD99 antigen	0.566 (0.515 to 0.622)	0.105
CDC14 cell division cycle 14 homolog A (S. cerevisiae)	1.203 (0.774 to 2.837)	0.709
CDC14 cell division cycle 14 homolog B (S. cerevisiae)	1.074 (1.006 to 1.12)	0.164
CDC23 (cell division cycle 23, yeast, homolog)	1.268 (0.983 to 1.866)	0.209
CDC28 protein kinase regulatory subunit 2	1.015 (0.993 to 1.038)	0.615
CDC42 binding protein kinase alpha (DMPK-like)	1.034 (0.735 to 1.251)	0.863
CDC45 cell division cycle 45-like (S. cerevisiae)	2.145 (1.768 to 2.602)	0.158
CDC-like kinase 1	0.897 (0.574 to 1.49)	0.732
CDC-like kinase 2	1.035 (0.939 to 1.29)	0.574
CDC-like kinase 3	1.209 (0.832 to 1.757)	0.701
CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 1	1.271 (0.762 to 2.483)	0.302
CDW52 antigen (CAMPATH-1 antigen)	0.683 (0.505 to 0.927)	0.0692
cell death-inducing DFFA-like effector a	1.144 (1.021 to 1.281)	0.447
cell division cycle 25B	1.136 (0.876 to 1.679)	0.302
cell division cycle 42 (GTP binding protein, 25kDa)	0.612	no replicates
cell matrix adhesion regulator	0.975 (0.754 to 1.259)	0.936
cellular retinoic acid binding protein 1	1.427 (0.785 to 2.695)	0.238
centrin, EF-hand protein, 1	1.958 (1.192 to 3.053)	0.071
centrin, EF-hand protein, 2	1.086 (0.627 to 2.511)	0.799
centrin, EF-hand protein, 3 (CDC31 homolog, yeast)	1.274	no replicates
centromere protein A, 17kDa	0.997 (0.771 to 1.15)	0.982
centromere protein E, 312kDa	1.363 (1.057 to 1.757)	0.437
centromere protein F, 350/400ka (mitosin)	0.931 (0.524 to 1.806)	0.798
cerebellar degeneration-related protein 1, 34kDa	0.673	no replicates
cerebellin 1 precursor	0.948 (0.695 to 1.223)	0.775
cerebral cavernous malformations 1	0.315	no replicates
ceroid-lipofuscinosis, neuronal 3, juvenile (Batten, Spielmeyer-Vogt disease)	1.333 (0.972 to 1.827)	0.53
ceroid-lipofuscinosis, neuronal 5	0.721 (0.505 to 0.922)	0.215
ceruloplasmin (ferroxidase)	0.372 (0.156 to 0.891)	0.461
c-fos induced growth factor (vascular endothelial growth factor D)	1.226 (0.777 to 3.345)	0.484
chaperonin containing TCP1, subunit 3 (gamma)	1.269 (1.218 to 1.322)	0.109
chaperonin containing TCP1, subunit 6A (zeta 1)	1.528	no replicates
Charot-Leyden crystal protein	0.975 (0.543 to 1.438)	0.91
checkpoint suppressor 1	0.775	no replicates
Chediak-Higashi syndrome 1	0.769 (0.728 to 0.811)	0.128
chemokine (C motif) ligand 1	1.08 (0.875 to 1.334)	0.776
chemokine (C motif) ligand 2	1.076 (0.934 to 1.239)	0.696
chemokine (C motif) receptor 1	1	no replicates
chemokine (C-C motif) ligand 11	1.218 (0.685 to 1.839)	0.574
chemokine (C-C motif) ligand 13	1.331 (1.277 to 1.387)	0.0919
chemokine (C-C motif) ligand 14	0.799 (0.711 to 0.898)	0.304
chemokine (C-C motif) ligand 15	0.801 (0.285 to 1.418)	0.583
chemokine (C-C motif) ligand 16	1.055 (0.649 to 1.604)	0.746
chemokine (C-C motif) ligand 17	0.936 (0.571 to 1.536)	0.916
chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	1.032 (0.594 to 1.833)	0.876
chemokine (C-C motif) ligand 19	0.653 (0.489 to 0.873)	0.38
chemokine (C-C motif) ligand 2	1.002 (0.967 to 1.038)	0.966
chemokine (C-C motif) ligand 20	0.821 (0.802 to 0.841)	0.0751
chemokine (C-C motif) ligand 21	0.976 (0.916 to 1.04)	0.768
chemokine (C-C motif) ligand 22	0.882	no replicates
chemokine (C-C motif) ligand 24	0.83 (0.388 to 1.536)	0.689
chemokine (C-C motif) ligand 25	1.021 (0.8 to 1.303)	0.946
chemokine (C-C motif) ligand 3	0.954 (0.725 to 1.087)	0.652
chemokine (C-C motif) ligand 8	0.734 (0.304 to 1.267)	0.271
chemokine (C-C motif) receptor 3	0.764 (0.413 to 1.288)	0.3
chemokine (C-C motif) receptor 4	1.187 (1.153 to 1.222)	0.107

chemokine (C-C motif) receptor 5	1.378 (1.087 to 1.748)	0.406
chemokine (C-C motif) receptor 6	0.604 (0.365 to 1.247)	0.308
chemokine (C-C motif) receptor 7	1.088 (0.659 to 1.738)	0.746
chemokine (C-C motif) receptor 8	1.272 (0.61 to 2.966)	0.43
chemokine (C-X3-C motif) ligand 1	1.354 (0.887 to 2.069)	0.604
chemokine (C-X3-C motif) receptor 1	1.224 (1.134 to 1.309)	0.0398
chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	1.01 (0.826 to 1.158)	0.933
chemokine (C-X-C motif) ligand 11	1.486 (1.374 to 1.607)	0.124
chemokine (C-X-C motif) ligand 2	0.928 (0.707 to 1.513)	0.631
chemokine (C-X-C motif) ligand 3	1.358 (1.247 to 1.479)	0.173
chemokine (C-X-C motif) ligand 5	0.81 (0.569 to 1.151)	0.656
chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	0.815 (0.469 to 1.178)	0.542
chemokine (C-X-C motif) receptor 3	0.984 (0.603 to 1.412)	0.929
chemokine binding protein 2	1.201 (0.912 to 1.608)	0.38
chemokine-like receptor 1	1.149 (0.731 to 2.399)	0.744
chimerin (chimaerin) 1	0.877 (0.63 to 1.052)	0.512
chimerin (chimaerin) 2	1.275 (0.682 to 2.458)	0.454
chitinase 1 (chitotriosidase)	0.967 (0.89 to 1.051)	0.754
chitinase 3-like 1 (cartilage glycoprotein-39)	0.729 (0.414 to 0.977)	0.205
chitobiase, di-N-acetyl-	1.173 (0.891 to 1.392)	0.115
CHK1 checkpoint homolog (S. pombe)	0.988 (0.729 to 1.228)	0.921
chloride channel 1, skeletal muscle (Thomsen disease, autosomal dominant)	1.039	no replicates
chloride channel 2	1.554 (0.541 to 3.153)	0.37
chloride channel 3	1.356 (1.092 to 1.684)	0.394
chloride channel 4	1.93	no replicates
chloride channel 6	0.942 (0.446 to 1.627)	0.892
chloride channel Ka	0.644	no replicates
chloride channel Kb	2.606 (1.16 to 5.854)	0.447
chloride channel, calcium activated, family member 1	1.18 (1.039 to 1.341)	0.418
chloride intracellular channel 1	0.807 (0.717 to 0.918)	0.096
chloride intracellular channel 2	0.862 (0.687 to 1.059)	0.357
cholecystokinin	0.548 (0.309 to 0.97)	0.483
cholecystokinin A receptor	1.023 (0.674 to 1.228)	0.885
cholecystokinin B receptor	1.042	no replicates
choline kinase	1.507 (1.478 to 1.536)	0.0303
choline kinase-like	0.994 (0.709 to 1.393)	0.988
cholinergic receptor, muscarinic 1	1.147 (1.012 to 1.3)	0.472
cholinergic receptor, muscarinic 2	0.981 (0.476 to 1.746)	0.936
cholinergic receptor, muscarinic 3	2.417 (1.305 to 4.477)	0.388
cholinergic receptor, muscarinic 4	0.426 (0.185 to 0.981)	0.493
cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	1.268 (1 to 2.119)	0.273
cholinergic receptor, nicotinic, alpha polypeptide 2 (neuronal)	1.024 (0.866 to 1.211)	0.91
cholinergic receptor, nicotinic, alpha polypeptide 3	0.961 (0.694 to 1.412)	0.808
cholinergic receptor, nicotinic, alpha polypeptide 5	0.557 (0.532 to 0.611)	0.00605
cholinergic receptor, nicotinic, alpha polypeptide 7	0.762 (0.606 to 0.874)	0.144
cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)	1.251 (0.79 to 1.979)	0.711
cholinergic receptor, nicotinic, beta polypeptide 3	5.239 (3.665 to 7.49)	0.135
cholinergic receptor, nicotinic, delta polypeptide	1.195 (0.852 to 2.105)	0.324
cholinergic receptor, nicotinic, epsilon polypeptide	0.941 (0.768 to 1.102)	0.627
cholinergic receptor, nicotinic, gamma polypeptide	1.037 (0.819 to 1.241)	0.797
chondroadherin	1.089 (0.703 to 1.752)	0.778
chondroitin sulfate proteoglycan 2 (versican)	1.141 (0.937 to 1.424)	0.265
chondroitin sulfate proteoglycan 3 (neurocan)	1.37 (0.778 to 2.266)	0.417
chondroitin sulfate proteoglycan 4 (melanoma-associated)	0.699 (0.436 to 1.171)	0.209
chorionic gonadotropin, beta polypeptide	0.829 (0.59 to 1.135)	0.242
chorionic somatomammotropin hormone 1 (placental lactogen)	0.895 (0.462 to 1.549)	0.694
choroideremia-like (Rab escort protein 2)	1.619 (1.073 to 3.253)	0.146
chromobox homolog 4 (Pc class homolog, Drosophila)	0.982 (0.873 to 1.105)	0.903
chromodomain helicase DNA binding protein 1	1.386	no replicates
chromodomain helicase DNA binding protein 2	1.008 (0.993 to 1.024)	0.676
chromogranin A (parathyroid secretory protein 1)	0.726 (0.451 to 1.058)	0.233
chromogranin B (secretogranin 1)	0.809 (0.562 to 1.022)	0.369
chromosome 1 open reading frame 1	1.081 (0.394 to 5.606)	0.87
chromosome 11 open reading frame 13	1.293 (0.518 to 2.174)	0.474
chromosome 11 open reading frame 8	1.338 (0.97 to 2.019)	0.0864
chromosome 16 open reading frame 3	1.308 (1.143 to 1.496)	0.296
chromosome 16 open reading frame 7	2.472 (1.085 to 5.636)	0.47
chromosome 18 open reading frame 1	1.743 (1.43 to 2.651)	0.0298

chromosome 19 open reading frame 2	0.935 (0.734 to 1.193)	0.829
chromosome 21 open reading frame 2	1.016 (0.807 to 1.28)	0.956
chromosome 21 open reading frame 4	0.744 (0.648 to 0.887)	0.0854
chromosome 22 open reading frame 1	1.082 (0.754 to 1.549)	0.629
chromosome 22 open reading frame 19	0.935 (0.898 to 0.992)	0.158
chromosome 4 open reading frame 6	1.196 (0.992 to 1.442)	0.514
chromosome 4 open reading frame 8	1.433 (1.264 to 1.625)	0.214
chromosome 8 open reading frame 1	0.959 (0.895 to 1.028)	0.652
chromosome condensation 1	0.978 (0.929 to 1.03)	0.741
chromosome condensation 1-like	1.723 (0.963 to 4.072)	0.341
chromosome X open reading frame 12	0.947 (0.495 to 1.496)	0.886
chromosome X open reading frame 2	0.762 (0.708 to 0.836)	0.0308
chymase 1, mast cell	1.145 (1.006 to 1.303)	0.485
chymotrypsin-like	0.968 (0.938 to 0.998)	0.482
chymotrypsinogen B1	0.317 (0.225 to 0.446)	0.185
clathrin, heavy polypeptide-like 1	0.906 (0.707 to 1.162)	0.76
clathrin, light polypeptide (Lca)	1.263 (0.781 to 2.443)	0.337
clathrin, light polypeptide (Lcb)	0.607 (0.393 to 0.801)	0.15
claudin 11 (oligodendrocyte transmembrane protein)	1.288	no replicates
claudin 3	0.901 (0.776 to 1.046)	0.612
claudin 5 (transmembrane protein deleted in velocardiofacial syndrome)	0.812 (0.773 to 0.852)	0.146
cleavage stimulation factor, 3' pre-RNA, subunit 1, 50kDa	1.053 (0.857 to 1.318)	0.644
cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa	3.662 (0.952 to 18.76)	0.17
cleavage stimulation factor, 3' pre-RNA, subunit 3, 77kDa	1.164 (1.023 to 1.255)	0.144
cleft lip and palate associated transmembrane protein 1	0.939 (0.787 to 1.341)	0.574
coagulation factor C homolog, cochlin (Limulus polyphemus)	0.545 (0.39 to 0.763)	0.322
coagulation factor II (thrombin)	1.674 (1.169 to 2.43)	0.135
coagulation factor II (thrombin) receptor	1.036 (0.787 to 1.761)	0.905
coagulation factor II (thrombin) receptor-like 1	1.162 (0.752 to 2.098)	0.456
coagulation factor II (thrombin) receptor-like 2	1.277 (0.995 to 1.638)	0.506
coagulation factor III (thromboplastin, tissue factor)	0.786 (0.407 to 1.152)	0.543
coagulation factor IX (plasma thromboplastic component, Christmas disease, hemophilia B)	1.192 (0.59 to 2.393)	0.583
coagulation factor V (proaccelerin, labile factor)	0.598 (0.341 to 1.538)	0.392
coagulation factor VII (serum prothrombin conversion accelerator)	1.164 (1.029 to 1.316)	0.433
coagulation factor VIII, procoagulant component (hemophilia A)	1.021 (0.885 to 1.177)	0.909
coagulation factor X	1.605 (1 to 5.412)	0.329
coagulation factor XI (plasma thromboplastin antecedent)	1.743 (1.072 to 2.833)	0.457
coagulation factor XII (Hageman factor)	0.996 (0.879 to 1.227)	0.971
coagulation factor XIII, A1 polypeptide	1.023 (0.663 to 1.638)	0.94
Cockayne syndrome 1 (classical)	0.906 (0.818 to 1.004)	0.512
cofilin 1 (non-muscle)	0.96 (0.719 to 1.308)	0.767
coilin	1.357 (1.062 to 1.98)	0.128
cold inducible RNA binding protein	0.459 (0.229 to 0.92)	0.464
colipase, pancreatic	1.006 (0.671 to 1.537)	0.972
collagen, type I, alpha 1	0.931 (0.805 to 1.077)	0.71
collagen, type IV, alpha 4	0.688 (0.392 to 1.208)	0.627
collagen, type IV, alpha 5 (Alport syndrome)	2.245 (1 to 5.041)	0.5
collagen, type IX, alpha 1	1.228 (1.138 to 1.326)	0.226
collagen, type IX, alpha 3	0.878 (0.453 to 1.702)	0.877
collagen, type V, alpha 1	0.57 (0.195 to 1.666)	0.693
collagen, type V, alpha 2	1.075 (0.801 to 1.275)	0.551
collagen, type VI, alpha 3	0.924 (0.612 to 1.286)	0.551
collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)	1.619 (1.001 to 3.872)	0.385
collagen, type X, alpha 1(Schmid metaphyseal chondrodysplasia)	1.139 (1.092 to 1.188)	0.2
collagen, type XII, alpha 1	1.354 (0.776 to 3.208)	0.395
collagen, type XIII, alpha 1	1.542 (0.803 to 2.735)	0.144
collagen, type XV, alpha 1	1.216 (1.214 to 1.217)	0.00372
collagen, type XVII, alpha 1	0.92 (0.829 to 1.02)	0.567
collapsin response mediator protein 1	0.793 (0.416 to 1.29)	0.562
colony stimulating factor 1 (macrophage)	1.137 (1.118 to 1.156)	0.0829
colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	0.724 (0.482 to 1.107)	0.124
colony stimulating factor 3 receptor (granulocyte)	1.002 (0.8 to 1.254)	0.995
complement component (3d/Epstein Barr virus) receptor 2	0.975 (0.751 to 1.266)	0.939
complement component 1, q subcomponent binding protein	1.282 (0.952 to 1.728)	0.557
complement component 1, r subcomponent	1.156	no replicates
complement component 1, s subcomponent	1.796 (1.145 to 2.555)	0.132
complement component 2	0.902 (0.66 to 1.56)	0.743

complement component 3	1.203 (1.007 to 1.437)	0.488
complement component 3a receptor 1	0.691 (0.252 to 1.095)	0.232
complement component 4 binding protein, alpha	0.818 (0.574 to 1.165)	0.671
complement component 4 binding protein, beta	0.951 (0.855 to 1.058)	0.719
complement component 4B	0.793 (0.697 to 0.901)	0.321
complement component 7	2.146 (1.094 to 4.207)	0.46
complement component 8, alpha polypeptide	0.851 (0.75 to 0.966)	0.423
complement component 8, beta polypeptide	1.196 (1.091 to 1.311)	0.301
complement component 8, gamma polypeptide	1.108 (0.922 to 1.309)	0.283
complement component 9	1.044 (0.384 to 2.804)	0.923
cone-rod homeobox	0.736 (0.664 to 0.816)	0.206
connective tissue growth factor	1.142 (0.951 to 1.438)	0.388
conserved helix-loop-helix ubiquitous kinase	0.926 (0.856 to 1.001)	0.506
contactin associated protein 1	0.909 (0.609 to 1.122)	0.68
COP9 constitutive photomorphogenic homolog subunit 3 (Arabidopsis)	1.308 (0.82 to 2.021)	0.241
coproporphyrinogen oxidase (coproporphyria, harderoporphyria)	0.981	no replicates
core promoter element binding protein	1.017	no replicates
core-binding factor, runt domain, alpha subunit 2; translocated to, 1; cyclin D-related	1.125 (0.882 to 1.392)	0.405
core-binding factor, runt domain, alpha subunit 2; translocated to, 3	1.221 (0.911 to 1.642)	0.202
coronin, actin binding protein, 2A	1.54 (1.008 to 2.757)	0.0782
corticotropin releasing hormone	0.909 (0.641 to 1.359)	0.628
corticotropin releasing hormone binding protein	1.356 (0.983 to 1.885)	0.247
corticotropin releasing hormone receptor 1	0.912 (0.399 to 1.561)	0.704
corticotropin releasing hormone receptor 2	0.839 (0.579 to 1.144)	0.471
cortistatin	0.884	no replicates
COX10 homolog, cytochrome c oxidase assembly protein, heme A: farnesyltransferase (yeast)	1.344 (0.713 to 2.68)	0.521
COX11 homolog, cytochrome c oxidase assembly protein (yeast)	1.069 (0.669 to 1.497)	0.724
coxsackie virus and adenovirus receptor	1.125 (0.802 to 1.702)	0.425
craniofacial development protein 1	0.845 (0.485 to 1.495)	0.546
creatine kinase, mitochondrial 2 (sarcomeric)	0.968 (0.948 to 0.988)	0.362
creatine kinase, muscle	0.942 (0.847 to 1.049)	0.677
CREB binding protein (Rubinstein-Taybi syndrome)	1.235 (0.795 to 2.024)	0.454
cryptochrome 1 (photolyase-like)	1.061 (0.465 to 2.419)	0.955
crystallin, alpha B	0.819 (0.725 to 0.924)	0.346
crystallin, beta A1	0.824 (0.572 to 1.089)	0.416
crystallin, beta A4	1.433 (1.062 to 1.933)	0.442
crystallin, beta B1	1.613 (1.027 to 3.043)	0.104
crystallin, beta B2	0.743 (0.518 to 1.161)	0.336
crystallin, gamma B	1.526 (1.189 to 1.811)	0.0802
crystallin, mu	35.98	no replicates
c-src tyrosine kinase	1.174 (0.921 to 1.495)	0.628
C-terminal binding protein 1	1.298 (0.697 to 2.539)	0.409
C-terminal binding protein 2	1.011 (0.824 to 1.154)	0.923
CTP synthase	0.761 (0.453 to 1.096)	0.413
cubilin (intrinsic factor-cobalamin receptor)	1.116 (1.021 to 1.22)	0.433
cullin 1	1.38 (1 to 1.793)	0.201
cullin 2	0.748 (0.599 to 0.934)	0.416
cullin 3	0.507 (0.0543 to 1.778)	0.605
cullin 5	0.715 (0.614 to 0.832)	0.271
cyclic AMP phosphoprotein, 19 kD	1.076 (0.885 to 1.307)	0.771
cyclic nucleotide gated channel alpha 1	0.434 (0.0464 to 1.791)	0.538
cyclic nucleotide gated channel alpha 3	0.607 (0.0418 to 1.894)	0.619
cyclic nucleotide gated channel beta 1	0.847	no replicates
cyclin F	1.059 (0.783 to 1.488)	0.789
cyclin G associated kinase	1.503 (1.264 to 1.787)	0.256
cyclin T1	1.546 (0.913 to 2.249)	0.106
cyclin T2	1.214 (1.113 to 1.324)	0.268
cyclin-dependent kinase 3	1.212 (0.997 to 1.473)	0.504
cyclin-dependent kinase 8	1.137 (0.922 to 1.321)	0.189
cyclin-dependent kinase inhibitor 1B (p27, Kip1)	1.25 (0.924 to 1.69)	0.595
cyclin-dependent kinase inhibitor 1C (p57, Kip2)	0.883 (0.487 to 1.503)	0.628
cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	0.936 (0.644 to 1.185)	0.671
cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)	1.616 (1.384 to 2.033)	0.0547
cyclin-dependent kinase-like 5	1 (0.897 to 1.114)	1
cystathionase (cystathionine gamma-lyase)	0.779 (0.468 to 1.073)	0.436
cystathionine-beta-synthase	0.898 (0.773 to 1.031)	0.162
cystatin A (stefin A)	1.48 (1.469 to 1.49)	0.0114
cystatin B (stefin B)	1.011 (0.907 to 1.127)	0.937

cystatin C (amyloid angiopathy and cerebral hemorrhage)	1.248 (1.113 to 1.339)	0.0618
cystatin D	1.082 (1.065 to 1.1)	0.131
cystatin E/M	0.69 (0.499 to 1.017)	0.0891
cystatin S	1.039 (0.774 to 1.286)	0.824
cystatin SA	0.871 (0.62 to 1.104)	0.353
cystatin SN	1.221 (0.797 to 1.872)	0.722
cysteine and glycine-rich protein 2	1.316	no replicates
cysteine and glycine-rich protein 3 (cardiac LIM protein)	0.814 (0.58 to 1.143)	0.653
cysteine conjugate-beta lyase; cytoplasmic (glutamine transaminase K, kyneurenine aminotransferase)	2.007 (1.111 to 3.623)	0.448
cysteine dioxygenase, type I	1.408 (0.838 to 2.803)	0.19
cysteine-rich protein 1 (intestinal)	0.771 (0.497 to 1.067)	0.205
cysteine-rich protein 2	0.909 (0.607 to 1.188)	0.55
cystinosis, nephropathic	1.836 (1.284 to 2.625)	0.338
cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMP-N-acetylneuraminate monooxygenase)	1.277 (1.015 to 1.906)	0.348
cytochrome b-245, beta polypeptide (chronic granulomatous disease)	1.34 (1.13 to 1.588)	0.335
cytochrome c oxidase subunit IV isoform 1	0.785 (0.474 to 1.027)	0.44
cytochrome c oxidase subunit VIa polypeptide 2	0.753 (0.538 to 1.261)	0.127
cytochrome c oxidase subunit VIb	1.415 (0.975 to 4.134)	0.271
cytochrome c oxidase subunit VIc	1.343 (1.289 to 1.399)	0.0881
cytochrome c oxidase subunit VIIa polypeptide 1 (muscle)	0.845 (0.686 to 0.969)	0.253
cytochrome c oxidase subunit VIIa polypeptide 2 (liver)	1.018 (0.964 to 1.075)	0.796
cytochrome c oxidase subunit VIIb	0.922 (0.641 to 1.325)	0.651
cytochrome c oxidase subunit VIII	1.085 (0.669 to 2.523)	0.754
cytochrome c-1	0.326 (0.0198 to 1.036)	0.319
cytochrome P450, family 1, subfamily A, polypeptide 1	1.017 (0.823 to 1.371)	0.892
cytochrome P450, family 1, subfamily A, polypeptide 2	0.962	no replicates
cytochrome P450, family 11, subfamily B, polypeptide 1	0.989 (0.758 to 1.154)	0.897
cytochrome P450, family 11, subfamily B, polypeptide 2	1.078 (0.833 to 1.234)	0.458
cytochrome P450, family 17, subfamily A, polypeptide 1	1.099 (1.016 to 1.188)	0.443
cytochrome P450, family 19, subfamily A, polypeptide 1	2.547 (0.958 to 6.772)	0.514
cytochrome P450, family 2, subfamily A, polypeptide 6	0.976 (0.797 to 1.17)	0.764
cytochrome P450, family 2, subfamily A, polypeptide 7	1.045 (0.927 to 1.331)	0.526
cytochrome P450, family 2, subfamily C, polypeptide 19	0.997 (0.622 to 1.324)	0.986
cytochrome P450, family 2, subfamily C, polypeptide 8	1.177 (0.715 to 2.462)	0.582
cytochrome P450, family 2, subfamily D, polypeptide 6	1.35 (1.182 to 1.54)	0.264
cytochrome P450, family 2, subfamily J, polypeptide 2	1.011 (0.805 to 1.304)	0.905
cytochrome P450, family 24, subfamily A, polypeptide 1	0.863 (0.417 to 1.617)	0.744
cytochrome P450, family 26, subfamily A, polypeptide 1	1.144 (0.704 to 2.335)	0.603
cytochrome P450, family 27, subfamily A, polypeptide 1	0.728 (0.517 to 1.025)	0.524
cytochrome P450, family 3, subfamily A, polypeptide 4	0.955 (0.865 to 1.053)	0.718
cytochrome P450, family 3, subfamily A, polypeptide 5	1.578 (1.096 to 2.27)	0.429
cytochrome P450, family 3, subfamily A, polypeptide 7	1.045 (0.975 to 1.122)	0.641
cytochrome P450, family 4, subfamily A, polypeptide 11	1.2 (0.961 to 1.368)	0.244
cytochrome P450, family 4, subfamily F, polypeptide 3	1.019 (0.626 to 1.582)	0.928
cytochrome P450, family 51, subfamily A, polypeptide 1	0.748 (0.43 to 1.207)	0.195
cytochrome P450, family 7, subfamily A, polypeptide 1	1.066 (0.882 to 1.289)	0.792
cytokine-inducible kinase	1.141 (1.031 to 1.262)	0.416
cytoplasmic linker 2	1.309 (1.201 to 1.552)	0.00603
cytosolic ovarian carcinoma antigen 1	0.861 (0.739 to 1.003)	0.506
cytotoxic T-lymphocyte-associated protein 4	0.828 (0.364 to 1.289)	0.691
D component of complement (adipsin)	1.285 (0.999 to 1.654)	0.501
D4, zinc and double PHD fingers family 1	0.974 (0.662 to 1.232)	0.904
dachshund homolog (Drosophila)	1.175 (0.909 to 1.924)	0.409
damage-specific DNA binding protein 1, 127kDa	0.898 (0.795 to 1.064)	0.187
damage-specific DNA binding protein 2, 48kDa	0.459 (0.0587 to 1.807)	0.534
D-amino-acid oxidase	1.475 (1.448 to 1.503)	0.03
D-aspartate oxidase	1.637	no replicates
dCMP deaminase	0.83 (0.772 to 0.893)	0.236
D-dopachrome tautomerase	1.316 (0.959 to 1.805)	0.545
DEAD (Asp-Glu-Ala-Asp) box polypeptide 1	0.865 (0.777 to 0.925)	0.114
DEAD (Asp-Glu-Ala-Asp) box polypeptide 10	0.997 (0.759 to 1.309)	0.992
DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	1.275 (0.969 to 1.583)	0.124
DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	1.361 (1.053 to 1.759)	0.442
DEAD (Asp-Glu-Ala-Asp) box polypeptide 6	1.161 (1.052 to 1.227)	0.0217
dead ringer-like 1 (Drosophila)	1.026 (0.961 to 1.096)	0.762
DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, S. cerevisiae)	0.71 (0.609 to 0.829)	0.271
deafness, autosomal dominant 5	1.174 (0.684 to 2.039)	0.481

DEAH (Asp-Glu-Ala-His) box polypeptide 15	1.5 (1.127 to 1.995)	0.391
DEAH (Asp-Glu-Ala-His) box polypeptide 16	0.895 (0.454 to 1.342)	0.777
DEAH (Asp-Glu-Ala-His) box polypeptide 8	0.92 (0.553 to 1.246)	0.669
DEAH (Asp-Glu-Ala-His) box polypeptide 9	1.085 (0.866 to 1.368)	0.523
death-associated protein	0.861 (0.586 to 1.094)	0.523
death-associated protein kinase 1	1.376 (1.242 to 1.524)	0.197
death-associated protein kinase 3	1.229 (0.871 to 1.589)	0.259
dedicator of cyto-kinesis 1	1.08 (0.912 to 1.47)	0.669
defensin, alpha 1, myeloid-related sequence	0.832 (0.715 to 0.915)	0.0527
defensin, alpha 4, corticostatin	1.27 (1.02 to 1.581)	0.472
defensin, alpha 6, Paneth cell-specific	1.022 (0.779 to 1.321)	0.85
defensin, beta 1	0.961 (0.808 to 1.336)	0.753
degenerative spermatocyte homolog, lipid desaturase (Drosophila)	0.753 (0.377 to 1.19)	0.504
deiodinase, iodothyronine, type I	1.085 (0.964 to 1.347)	0.529
deiodinase, iodothyronine, type II	1.622 (1.174 to 2.34)	0.137
deiodinase, iodothyronine, type III	0.915 (0.848 to 0.988)	0.453
DEK oncogene (DNA binding)	2.256 (1.504 to 3.385)	0.294
deleted in azoospermia	1.143 (0.908 to 1.44)	0.665
deleted in azoospermia-like	0.448 (0.248 to 0.808)	0.404
deleted in malignant brain tumors 1	0.837 (0.666 to 1.583)	0.464
delta sleep inducing peptide, immunoreactor	0.328	no replicates
delta-like 1 (Drosophila)	0.824 (0.46 to 1.474)	0.795
deltex homolog 1 (Drosophila)	1.227 (1.03 to 1.462)	0.45
density-regulated protein	1.331 (1.012 to 1.607)	0.178
dentin matrix acidic phosphoprotein	0.886 (0.483 to 1.469)	0.651
deoxycytidine kinase	1.307 (0.936 to 1.586)	0.252
deoxyhypusine synthase	1.133 (0.898 to 1.353)	0.243
deoxynucleotidyltransferase, terminal	0.703 (0.431 to 0.962)	0.138
deoxyribonuclease I-like 2	1.311 (0.83 to 2.677)	0.292
deoxyribonuclease I-like 3	1.094 (0.773 to 1.547)	0.839
dermatan sulfate proteoglycan 3	1.321 (0.935 to 1.968)	0.0948
dermatopontin	0.845 (0.5 to 1.04)	0.409
desmin	1.059 (0.956 to 1.307)	0.488
desmoglein 3 (pemphigus vulgaris antigen)	1.153 (0.984 to 1.798)	0.406
destrin (actin depolymerizing factor)	0.721 (0.584 to 0.891)	0.366
developmentally regulated GTP binding protein 1	1.56 (1.554 to 1.566)	0.00551
developmentally regulated GTP binding protein 2	0.829 (0.643 to 1.031)	0.306
diacylglycerol kinase, delta 130kDa	0.596 (0.327 to 1.088)	0.548
diacylglycerol kinase, epsilon 64kDa	1.968 (0.92 to 4.485)	0.128
diacylglycerol kinase, gamma 90kDa	0.828 (0.808 to 0.85)	0.0852
diacylglycerol kinase, theta 110kDa	0.914 (0.868 to 0.963)	0.336
diacylglycerol kinase, zeta 104kDa	1.266 (1.009 to 1.587)	0.487
diaphanous homolog 1 (Drosophila)	0.883 (0.775 to 1.032)	0.277
diaphanous homolog 2 (Drosophila)	0.869 (0.609 to 1.409)	0.632
diaphorase (NADH) (cytochrome b-5 reductase)	1.411 (1.348 to 1.478)	0.0846
dihydrofolate reductase	3.349 (1.044 to 10.75)	0.488
dihydrolipoamide branched chain transacylase (E2 component of branched chain keto acid dehydrogenase complex; maple syrup urine disease)	0.941 (0.928 to 0.954)	0.14
dihydrolipoamide dehydrogenase (E3 component of pyruvate dehydrogenase complex, 2-oxo-glutarate complex, branched chain keto acid dehydrogenase complex)	0.844 (0.355 to 1.204)	0.603
dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex)	1.054 (0.77 to 1.57)	0.824
dihydropyrimidinase	0.638 (0.505 to 0.792)	0.0205
dihydropyrimidinase-like 2	0.861 (0.819 to 0.905)	0.205
dihydropyrimidinase-like 3	1.066 (0.977 to 1.164)	0.597
dihydropyrimidine dehydrogenase	1.235 (0.832 to 1.704)	0.421
dipeptidase 1 (renal)	0.878 (0.533 to 1.509)	0.587
dipeptidylpeptidase 6	1.154 (0.955 to 1.635)	0.321
diptheria toxin resistance protein required for diphthamide biosynthesis-like 1 (S. cerevisiae)	1.052 (0.898 to 1.232)	0.803
diptheria toxin resistance protein required for diphthamide biosynthesis-like 2 (S. cerevisiae)	0.734 (0.519 to 1.037)	0.535
disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)	1.302 (0.794 to 2.211)	0.2
discoidin domain receptor family, member 2	1.015 (0.926 to 1.112)	0.901
dishevelled, dsh homolog 1 (Drosophila)	1.569 (1.211 to 2.032)	0.332
dishevelled, dsh homolog 2 (Drosophila)	1.355 (1.025 to 1.582)	0.162
distal-less homeo box 2	0.829 (0.513 to 1.341)	0.763
distal-less homeo box 3	1.593 (1.001 to 2.535)	0.499
distal-less homeo box 5	1.271 (0.573 to 2.877)	0.658

distal-less homeo box 6	1.178 (1.005 to 1.381)	0.491
distal-less homeobox 4	1.063 (0.618 to 1.73)	0.732
Dmx-like 1	1.337 (1.075 to 1.663)	0.41
DNA (cytosine-5-)-methyltransferase 1	0.921 (0.855 to 0.993)	0.47
DNA (cytosine-5-)-methyltransferase 2	1.226 (1 to 1.467)	0.209
DNA (cytosine-5-)-methyltransferase 3 beta	1.105 (0.854 to 1.341)	0.365
DNA fragmentation factor, 45kDa, alpha polypeptide	0.868 (0.579 to 1.171)	0.33
DNA segment on chromosome 21 (unique) 2056 expressed sequence	0.87 (0.72 to 1.051)	0.225
DNA-damage-inducible transcript 3	1.205 (0.75 to 1.751)	0.533
DnaJ (Hsp40) homolog, subfamily A, member 1	1.039 (1.035 to 1.043)	0.0689
DnaJ (Hsp40) homolog, subfamily B, member 2	1.272 (1.025 to 1.498)	0.166
DnaJ (Hsp40) homolog, subfamily C, member 3	0.751 (0.577 to 0.978)	0.475
DnaJ (Hsp40) homolog, subfamily C, member 4	0.952 (0.816 to 1.21)	0.627
DnaJ (Hsp40) homolog, subfamily C, member 7	1.083 (0.605 to 2.041)	0.81
docking protein 1, 62kDa (downstream of tyrosine kinase 1)	1.186 (1.115 to 1.261)	0.22
dodecenoyl-Coenzyme A delta isomerase (3,2 trans-enoyl-Coenzyme A isomerase)	1.093 (0.924 to 1.292)	0.69
dolichyl-diphosphooligosaccharide-protein glycosyltransferase	0.889 (0.836 to 0.996)	0.175
dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase)	1.31 (1.044 to 1.649)	0.178
dolichyl-phosphate mannosyltransferase polypeptide 1, catalytic subunit	0.806 (0.26 to 1.384)	0.614
dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit	1.339 (0.967 to 2.486)	0.445
dom-3 homolog Z (C. elegans)	1.107 (0.799 to 2.465)	0.73
dopamine receptor D1	0.701 (0.36 to 1.366)	0.688
dopamine receptor D3	1.095 (0.715 to 1.677)	0.866
dopamine receptor D4	1.191 (0.863 to 2.369)	0.392
dopamine receptor D5	0.687 (0.362 to 1.026)	0.197
double C2-like domains, alpha	0.342	no replicates
double C2-like domains, beta	2.322 (1.205 to 5.025)	0.1
Down syndrome critical region gene 1	1.072 (0.629 to 1.835)	0.843
Down syndrome critical region gene 2	1.031 (0.823 to 1.291)	0.915
down-regulator of transcription 1, TBP-binding (negative cofactor 2)	0.86 (0.77 to 0.96)	0.401
drebrin 1	0.653 (0.49 to 0.923)	0.148
dual specificity phosphatase 1	0.984 (0.444 to 1.559)	0.958
dual specificity phosphatase 2	0.937 (0.912 to 0.962)	0.245
dual specificity phosphatase 3 (vaccinia virus phosphatase VH1-related)	1.149 (0.827 to 1.844)	0.522
dual specificity phosphatase 4	1.198 (1.049 to 1.369)	0.404
dual specificity phosphatase 5	1.003 (0.865 to 1.459)	0.985
dual specificity phosphatase 6	1.134 (0.374 to 4.799)	0.883
dual specificity phosphatase 8	1.059 (0.385 to 2.91)	0.867
dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	1.248 (0.922 to 3.039)	0.383
dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	1.52 (1.304 to 1.772)	0.224
dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3	2.627 (2.508 to 2.751)	0.0304
dUTP pyrophosphatase	1.244 (1.106 to 1.399)	0.314
dynactin 1 (p150, glued homolog, Drosophila)	1.859 (1.334 to 2.589)	0.313
dynamin 1	1.285 (0.771 to 4.144)	0.57
dynein, axonemal, light intermediate polypeptide 1	1.019 (0.802 to 1.296)	0.95
dynein, cytoplasmic, intermediate polypeptide 1	1.325 (1.173 to 1.497)	0.261
dynein, cytoplasmic, light intermediate polypeptide 2	0.911 (0.534 to 1.74)	0.812
dynein, cytoplasmic, light polypeptide 1	0.975 (0.952 to 0.998)	0.48
dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive)	1.086 (0.796 to 1.292)	0.649
dyskeratosis congenita 1, dyskerin	1	no replicates
dystrobrevin, alpha	1.246 (0.966 to 1.606)	0.546
dystroglycan 1 (dystrophin-associated glycoprotein 1)	1.124 (1.102 to 1.146)	0.107
dystrophia myotonica-protein kinase	0.99 (0.933 to 1.051)	0.897
dystrophin (muscular dystrophy, Duchenne and Becker types)	0.828 (0.818 to 0.838)	0.0406
dystrophin related protein 2	0.961 (0.762 to 1.211)	0.891
E2F transcription factor 2	1.287 (0.883 to 1.832)	0.354
E2F transcription factor 3	1.299 (0.632 to 2.209)	0.313
E2F transcription factor 4, p107/p130-binding	1.463 (0.769 to 2.562)	0.157
E4F transcription factor 1	1.241 (0.915 to 1.487)	0.068
E74-like factor 3 (ets domain transcription factor, epithelial-specific)	1.043 (1.031 to 1.055)	0.167
E74-like factor 4 (ets domain transcription factor)	1.091 (1.004 to 1.186)	0.485
E74-like factor 5 (ets domain transcription factor)	1.281 (0.926 to 2.779)	0.414
early endosome antigen 1, 162kD	1.299 (0.973 to 1.734)	0.532
early growth response 3	1	no replicates
early growth response 4	1.042 (0.841 to 1.293)	0.878
echinoderm microtubule associated protein like 1	0.994 (0.814 to 1.209)	0.961
ecotropic viral integration site 2B	0.994 (0.744 to 1.326)	0.986

ectodermal dysplasia 1, anhidrotic	2.154 (1.359 to 3.413)	0.344
ectodermal-neural cortex (with BTB-like domain)	1.508 (1.288 to 1.766)	0.233
ectonucleoside triphosphate diphosphohydrolase 1	0.923 (0.603 to 1.517)	0.795
ectonucleoside triphosphate diphosphohydrolase 2	0.845 (0.67 to 1.122)	0.381
ectonucleoside triphosphate diphosphohydrolase 3	1.443 (0.782 to 3.511)	0.505
ectonucleoside triphosphate diphosphohydrolase 5	1.147 (1.109 to 1.186)	0.152
ectonucleoside triphosphate diphosphohydrolase 6 (putative function)	0.902 (0.733 to 1.429)	0.561
ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	1.216 (0.693 to 2.146)	0.565
ectonucleotide pyrophosphatase/phosphodiesterase 3	1.47 (1.016 to 2.127)	0.487
EGF-containing fibulin-like extracellular matrix protein 1	1.276 (1.179 to 1.525)	0.0273
egf-like module containing, mucin-like, hormone receptor-like sequence 1	1.995	no replicates
elastase 2, neutrophil	1.337 (0.947 to 2.157)	0.215
elastase 2A	0.901 (0.378 to 2.222)	0.858
elastin (supravalvular aortic stenosis, Williams-Beuren syndrome)	1.108 (0.989 to 1.247)	0.264
ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R)	1.059 (0.904 to 1.155)	0.545
ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)	1.434 (0.791 to 4.092)	0.286
electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)	1.019 (0.96 to 1.081)	0.807
electron-transfer-flavoprotein, beta polypeptide	0.697 (0.386 to 0.956)	0.347
electron-transferring-flavoprotein dehydrogenase	0.62 (0.444 to 0.866)	0.388
ELK1, member of ETS oncogene family	0.974 (0.803 to 1.245)	0.788
ELK4, ETS-domain protein (SRF accessory protein 1)	1.446 (1.026 to 1.779)	0.0216
emerin (Emery-Dreifuss muscular dystrophy)	1.159 (0.815 to 1.398)	0.313
endoglin (Osler-Rendu-Weber syndrome 1)	1.093 (0.684 to 1.746)	0.881
endometrial bleeding associated factor (left-right determination, factor A; transforming growth factor beta superfamily)	0.801 (0.729 to 0.881)	0.258
endonuclease G	0.956 (0.607 to 1.673)	0.83
endosulfine alpha	0.904 (0.621 to 1.338)	0.564
endothelial differentiation, G-protein-coupled receptor 6	1.063 (1.06 to 1.065)	0.0236
endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 2	1.028 (0.982 to 1.076)	0.654
endothelial differentiation, sphingolipid G-protein-coupled receptor, 1	1.563 (0.723 to 3.905)	0.46
endothelial differentiation, sphingolipid G-protein-coupled receptor, 3	1.166 (0.721 to 1.885)	0.803
endothelial PAS domain protein 1	1.147 (1.118 to 1.176)	0.116
endothelin 1	1.399 (0.973 to 2.015)	0.119
endothelin converting enzyme 1	1.489 (1.282 to 1.729)	0.229
engrailed homolog 1	0.848 (0.481 to 1.2)	0.475
engrailed homolog 2	0.952 (0.748 to 1.446)	0.759
enhancer of rudimentary homolog (Drosophila)	1.038 (0.25 to 3.747)	0.951
enhancer of zeste homolog 1 (Drosophila)	1.073 (1.008 to 1.142)	0.461
enhancer of zeste homolog 2 (Drosophila)	0.97 (0.666 to 1.479)	0.873
enolase 2, (gamma, neuronal)	1.654 (0.723 to 5.876)	0.233
enolase 3, (beta, muscle)	0.876 (0.829 to 0.974)	0.131
enoyl Coenzyme A hydratase 1, peroxisomal	1.169 (0.974 to 1.384)	0.264
enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	0.747 (0.387 to 1.201)	0.345
enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	1.222 (0.817 to 1.577)	0.429
envoplakin	0.69 (0.566 to 0.844)	0.0201
EphA3	0.507 (0.209 to 1.233)	0.585
EphA4	0.911 (0.698 to 1.569)	0.655
EphA7	0.907 (0.531 to 1.436)	0.767
EphB3	0.978 (0.746 to 1.136)	0.887
EphB4	1.151 (1.06 to 1.297)	0.149
EphB6	0.717 (0.366 to 1.088)	0.43
ephrin-A2	0.953 (0.767 to 1.136)	0.609
ephrin-A3	0.863 (0.639 to 1.052)	0.436
ephrin-A4	0.906 (0.638 to 1.285)	0.562
epidermal growth factor (beta-urogastrone)	1.011 (0.983 to 1.04)	0.765
epidermal growth factor receptor pathway substrate 8	1.489 (1.177 to 2.09)	0.0586
epimorphin	1.06 (0.938 to 1.228)	0.306
epiregulin	0.921 (0.844 to 1.005)	0.518
epithelial membrane protein 1	0.892 (0.823 to 0.967)	0.39
epithelial membrane protein 2	1.154 (1.025 to 1.3)	0.44
epithelial membrane protein 3	1.018 (0.808 to 1.165)	0.89
epoxide hydrolase 1, microsomal (xenobiotic)	0.863 (0.752 to 1)	0.215
Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)	1.392 (0.662 to 2.929)	0.734
ER to nucleus signalling 1	0.812 (0.479 to 1.078)	0.514
erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)	2.546 (0.965 to 11.25)	0.341
erythrocyte membrane protein band 4.1-like 2	1.361	no replicates

erythrocyte membrane protein band 4.2	1.225 (0.997 to 1.506)	0.504
erythrocyte membrane protein band 4.9 (dematin)	1.505 (1.269 to 1.783)	0.251
erythropoietin	2.738 (2.533 to 2.961)	0.0492
estrogen receptor 1	0.642 (0.39 to 1.055)	0.536
estrogen-related receptor alpha	1.265 (1.139 to 1.405)	0.267
estrogen-related receptor beta	0.94 (0.681 to 1.522)	0.743
estrogen-related receptor gamma	0.911 (0.889 to 0.934)	0.165
ets variant gene 1	1.026 (0.64 to 1.911)	0.9
ets variant gene 5 (ets-related molecule)	2.257 (1.002 to 5.085)	0.499
Ets2 repressor factor	1.148 (0.998 to 1.322)	0.505
eukaryotic translation elongation factor 1 alpha 1	1.123 (1.044 to 1.209)	0.359
eukaryotic translation elongation factor 1 alpha 2	0.904 (0.519 to 1.398)	0.583
eukaryotic translation elongation factor 1 beta 2	1.413 (1.41 to 1.417)	0.0051
eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)	1.479 (0.755 to 4.968)	0.294
eukaryotic translation elongation factor 1 gamma	1.039 (0.903 to 1.195)	0.831
eukaryotic translation elongation factor 2	1.464 (0.797 to 2.757)	0.231
eukaryotic translation initiation factor 1A	1.029 (0.96 to 1.103)	0.754
eukaryotic translation initiation factor 2, subunit 1 alpha, 35kDa	0.938 (0.805 to 1.094)	0.749
eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa	1.366 (1.129 to 1.604)	0.0932
eukaryotic translation initiation factor 2B, subunit 1 alpha, 26kDa	1.201 (0.948 to 1.368)	0.263
eukaryotic translation initiation factor 3, subunit 1 alpha, 35kDa	0.673 (0.351 to 1.293)	0.653
eukaryotic translation initiation factor 3, subunit 10 theta, 150/170kDa	0.653 (0.464 to 0.919)	0.43
eukaryotic translation initiation factor 3, subunit 2 beta, 36kDa	1.41 (0.941 to 2.114)	0.551
eukaryotic translation initiation factor 3, subunit 3 gamma, 40kDa	0.803 (0.583 to 1.106)	0.618
eukaryotic translation initiation factor 3, subunit 4 delta, 44kDa	1.457 (0.865 to 3.919)	0.527
eukaryotic translation initiation factor 3, subunit 5 epsilon, 47kDa	1.082 (0.869 to 1.347)	0.78
eukaryotic translation initiation factor 3, subunit 6 48kDa	1.124 (0.59 to 2.872)	0.75
eukaryotic translation initiation factor 3, subunit 7 zeta, 66/67kDa	0.838 (0.774 to 0.907)	0.269
eukaryotic translation initiation factor 3, subunit 8, 110kDa	0.945 (0.674 to 1.313)	0.798
eukaryotic translation initiation factor 3, subunit 9 eta, 116kDa	1.328 (1.17 to 1.506)	0.266
eukaryotic translation initiation factor 4 gamma, 1	1.285 (1.122 to 1.47)	0.315
eukaryotic translation initiation factor 4 gamma, 2	1.432 (1.377 to 1.489)	0.069
eukaryotic translation initiation factor 4 gamma, 3	1.313 (1.257 to 1.373)	0.102
eukaryotic translation initiation factor 4A, isoform 1	1.788 (1.081 to 4.058)	0.071
eukaryotic translation initiation factor 4A, isoform 2	1.198 (0.984 to 1.674)	0.134
eukaryotic translation initiation factor 4B	2.379 (1.908 to 3.584)	0.0517
eukaryotic translation initiation factor 4E	0.913 (0.827 to 0.983)	0.0921
eukaryotic translation initiation factor 4E binding protein 1	0.539 (0.34 to 0.854)	0.407
eukaryotic translation initiation factor 4E binding protein 2	1.285 (0.931 to 2.169)	0.291
eukaryotic translation initiation factor 4E binding protein 3	1.466 (1.342 to 1.568)	0.0143
eukaryotic translation initiation factor 5	1.151 (0.944 to 1.403)	0.608
eukaryotic translation initiation factor 5A	0.646 (0.431 to 1.296)	0.338
eukaryotic translation termination factor 1	0.971 (0.646 to 1.561)	0.896
eve, even-skipped homeo box homolog 1 (Drosophila)	1.112 (0.753 to 2.154)	0.678
Ewing sarcoma breakpoint region 1	1.302 (1.197 to 1.416)	0.196
excision repair cross-complementing rodent repair deficiency, complementation group 4	0.943 (0.915 to 0.973)	0.308
exostoses (multiple) 1	1.158 (1 to 1.389)	0.092
exostoses (multiple)-like 1	0.967 (0.919 to 1.017)	0.624
exostoses (multiple)-like 3	0.974 (0.869 to 1.093)	0.858
exportin 1 (CRM1 homolog, yeast)	1.847 (1.595 to 2.199)	0.0225
extracellular matrix protein 1	0.824 (0.399 to 2.078)	0.73
extracellular matrix protein 2, female organ and adipocyte specific	1.113 (0.875 to 1.415)	0.734
eyes absent homolog 1 (Drosophila)	1.108 (0.766 to 1.603)	0.827
eyes absent homolog 2 (Drosophila)	0.546 (0.381 to 0.757)	0.0936
eyes absent homolog 3 (Drosophila)	1.206 (1.099 to 1.324)	0.293
eyes absent homolog 4 (Drosophila)	1.04 (0.541 to 2.409)	0.886
faciogenital dysplasia (Aarskog-Scott syndrome)	1.077 (1.042 to 1.112)	0.263
Fanconi anemia, complementation group A	1.124 (0.924 to 1.368)	0.657
Fanconi anemia, complementation group C	0.901 (0.652 to 1.155)	0.603
Fanconi anemia, complementation group G	0.779 (0.604 to 1.104)	0.3
farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltranstransferase, geranyltranstransferase)	1.187 (0.884 to 1.553)	0.151
farnesyl-diphosphate farnesyltransferase 1	0.752 (0.545 to 1.038)	0.539
farnesyltransferase, CAAX box, alpha	0.942 (0.928 to 0.956)	0.157
Fas (TNFRSF6)-associated via death domain	1.136 (1.042 to 1.204)	0.102
fascin homolog 1, actin-bundling protein (Strongylocentrotus purpuratus)	0.969 (0.946 to 0.992)	0.41
FAT tumor suppressor homolog 1 (Drosophila)	1.181 (0.964 to 1.486)	0.168
fatty acid amide hydrolase	0.828 (0.573 to 1.196)	0.698

fatty acid binding protein 1, liver	1.087 (0.85 to 1.391)	0.791
fatty acid binding protein 2, intestinal	1.291 (0.483 to 4.679)	0.626
fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor)	1.432 (1.385 to 1.481)	0.0595
fatty acid binding protein 4, adipocyte	1.142 (0.952 to 1.37)	0.599
fatty acid binding protein 5 (psoriasis-associated)	1.072 (1.024 to 1.121)	0.368
fatty acid binding protein 6, ileal (gastrotropin)	0.996	no replicates
fatty acid binding protein 7, brain	0.969 (0.599 to 1.441)	0.835
fatty acid synthase	1.035 (0.682 to 1.571)	0.844
fatty-acid-Coenzyme A ligase, long-chain 1	1.309 (0.944 to 2.063)	0.37
fatty-acid-Coenzyme A ligase, long-chain 3	0.982 (0.93 to 1.037)	0.795
fatty-acid-Coenzyme A ligase, long-chain 4	0.957 (0.543 to 3.538)	0.906
FBJ murine osteosarcoma viral oncogene homolog B	1.207 (1.12 to 1.302)	0.241
Fc fragment of IgA, receptor for	1.178 (0.652 to 2.328)	0.471
Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide	1.011 (0.427 to 2.868)	0.979
Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	0.894 (0.694 to 1.054)	0.472
Fc fragment of IgE, low affinity II, receptor for (CD23A)	0.848 (0.598 to 1.351)	0.567
Fc fragment of IgG, low affinity IIb, receptor for (CD32)	1.001 (0.343 to 2)	0.998
Fc fragment of IgG, receptor, transporter, alpha	0.961 (0.555 to 1.198)	0.795
feline sarcoma oncogene	1.302 (1.115 to 1.52)	0.337
fer (fps/fes related) tyrosine kinase (phosphoprotein NCP94)	0.739 (0.38 to 1.148)	0.465
ferredoxin 1	1.08 (0.825 to 1.732)	0.68
ferredoxin reductase	1.003 (0.724 to 1.349)	0.985
ferritin, heavy polypeptide 1	0.772 (0.729 to 0.816)	0.136
ferritin, light polypeptide	2.752	no replicates
fetal Alzheimer antigen	1.002 (0.964 to 1.036)	0.925
fibrillarin	1.047 (0.955 to 1.148)	0.706
fibrillin 1 (Marfan syndrome)	0.771 (0.71 to 0.838)	0.196
fibrinogen, A alpha polypeptide	0.639 (0.461 to 0.887)	0.402
fibrinogen, gamma polypeptide	0.731	no replicates
fibrinogen-like 1	1.042 (0.767 to 1.356)	0.778
fibroblast activation protein, alpha	1.283 (1.009 to 1.631)	0.488
fibroblast growth factor 1 (acidic)	0.935 (0.733 to 1.124)	0.566
fibroblast growth factor 10	0.704 (0.367 to 1.353)	0.686
fibroblast growth factor 11	0.971 (0.73 to 1.146)	0.858
fibroblast growth factor 13	0.945 (0.877 to 1.018)	0.586
fibroblast growth factor 14	1.668 (0.877 to 3.002)	0.145
fibroblast growth factor 17	1.16 (0.993 to 1.413)	0.0601
fibroblast growth factor 18	0.922 (0.557 to 1.206)	0.778
fibroblast growth factor 2 (basic)	0.5	no replicates
fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome)	1.053 (0.879 to 1.261)	0.823
fibulin 1	1.311 (1.047 to 1.678)	0.0717
ficolin (collagen/fibrinogen domain containing lectin) 2 (hucolin)	1.01 (1 to 1.019)	0.487
ficolin (collagen/fibrinogen domain containing) 1	1.478	no replicates
filamin B, beta (actin binding protein 278)	1.133 (1.114 to 1.152)	0.0862
filamin C, gamma (actin binding protein 280)	0.847 (0.716 to 1.002)	0.503
Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed (fox derived); ribosomal protein S30	1.037 (0.67 to 1.779)	0.845
FK506 binding protein 1A, 12kDa	0.561 (0.45 to 0.699)	0.232
FK506 binding protein 1B, 12.6 kDa	1.042 (0.795 to 1.367)	0.903
FK506 binding protein 2, 13kDa	1.145 (0.619 to 1.666)	0.706
FK506 binding protein 3, 25kDa	1.074 (0.945 to 1.208)	0.42
FK506 binding protein 4, 59kDa	0.809 (0.36 to 1.287)	0.655
FK506 binding protein 5	1.13 (0.96 to 1.33)	0.591
FK506 binding protein 6, 36kDa	1.129 (1 to 1.381)	0.186
flap structure-specific endonuclease 1	1.288 (1.279 to 1.296)	0.0167
flavin containing monooxygenase 2	0.85 (0.7 to 1)	0.151
flavin containing monooxygenase 3	1.328 (0.993 to 2.047)	0.327
flavin containing monooxygenase 4	0.838 (0.495 to 1.258)	0.436
flavin containing monooxygenase 5	0.867 (0.754 to 0.998)	0.495
flightless I homolog (Drosophila)	0.872 (0.768 to 0.99)	0.476
flotillin 2	1.249 (0.955 to 1.634)	0.559
fms-related tyrosine kinase 4	0.893 (0.469 to 1.421)	0.66
folate receptor 1 (adult)	1.546	no replicates
folate receptor 3 (gamma)	0.926 (0.68 to 1.26)	0.844
follicle stimulating hormone, beta polypeptide	1.424 (0.673 to 3.006)	0.271
follicular lymphoma variant translocation 1	1.247 (1.183 to 1.314)	0.149
folylpolyglutamate synthase	1.508 (1.045 to 2.132)	0.184

forkhead box A1	0.98 (0.452 to 1.488)	0.964
forkhead box A3	1.151 (0.598 to 2.42)	0.675
forkhead box C2 (MFH-1, mesenchyme forkhead 1)	1.821	no replicates
forkhead box D1	0.895 (0.613 to 1.107)	0.616
forkhead box D2	0.881 (0.851 to 0.905)	0.0201
forkhead box F1	0.932 (0.851 to 1.021)	0.581
forkhead box F2	1.088 (1.062 to 1.113)	0.173
forkhead box G1A	0.994 (0.62 to 1.333)	0.972
forkhead box G1B	1.558 (1.531 to 1.585)	0.0248
forkhead box J1	0.927 (0.905 to 0.949)	0.193
forkhead box N1	1.213 (1.03 to 1.43)	0.448
forkhead box O3A	2.04 (1.925 to 2.163)	0.0521
formin-like	0.991	no replicates
formyl peptide receptor 1	1.012 (0.566 to 1.765)	0.949
formyl peptide receptor-like 1	1.131 (0.744 to 1.406)	0.45
formyl peptide receptor-like 2	1.129 (0.84 to 1.517)	0.752
FOS-like antigen 2	0.854 (0.473 to 1.178)	0.496
four and a half LIM domains 1	1.094 (0.941 to 1.272)	0.658
four and a half LIM domains 2	0.884 (0.771 to 1.026)	0.275
four and a half LIM domains 3	0.985 (0.886 to 1.096)	0.913
fragile histidine triad gene	0.888 (0.86 to 0.913)	0.00347
fragile X mental retardation 1	1.181 (1 to 1.591)	0.382
fragile X mental retardation 2	1.458 (0.818 to 4.014)	0.361
frizzled homolog 1 (Drosophila)	1.637 (1.38 to 2.278)	0.0966
frizzled homolog 5 (Drosophila)	1.27 (1.089 to 1.619)	0.191
frizzled homolog 6 (Drosophila)	1.437 (0.639 to 2.139)	0.157
frizzled homolog 7 (Drosophila)	0.836 (0.536 to 0.99)	0.189
frizzled homolog 9 (Drosophila)	1.057 (0.864 to 1.534)	0.691
fructose-1,6-bisphosphatase 1	1.535 (1.535 to 1.536)	0.000296
fructose-1,6-bisphosphatase 2	0.87 (0.503 to 1.131)	0.51
FSH primary response (LRPR1 homolog, rat) 1	1.237 (0.824 to 1.584)	0.17
FSHD region gene 1	1.082 (0.49 to 2.236)	0.817
fucose-1-phosphate guanylyltransferase	1.034	no replicates
fucosidase, alpha-L- 1, tissue	1.264 (1.073 to 1.548)	0.161
fucosyltransferase 1 (galactoside 2-alpha-L-fucosyltransferase, Bombay phenotype included)	1.241 (0.746 to 2.744)	0.399
fucosyltransferase 2 (secretor status included)	0.964 (0.8 to 1.162)	0.736
fucosyltransferase 3 (galactoside 3(4)-L-fucosyltransferase, Lewis blood group included)	1.09 (1.065 to 1.115)	0.166
fucosyltransferase 4 (alpha (1,3) fucosyltransferase, myeloid-specific)	1.073 (1.003 to 1.149)	0.487
fucosyltransferase 5 (alpha (1,3) fucosyltransferase)	0.88 (0.32 to 3.824)	0.88
fucosyltransferase 6 (alpha (1,3) fucosyltransferase)	0.821 (0.603 to 1.119)	0.639
fucosyltransferase 7 (alpha (1,3) fucosyltransferase)	1.296 (1.134 to 1.48)	0.302
fucosyltransferase 8 (alpha (1,6) fucosyltransferase)	1.182 (0.828 to 1.482)	0.18
fumarylacetoacetate hydrolase (fumarylacetoacetase)	0.865 (0.715 to 0.924)	0.04
furin (paired basic amino acid cleaving enzyme)	0.931 (0.65 to 1.144)	0.628
fusion, derived from t(12;16) malignant liposarcoma	1.887 (0.672 to 5.303)	0.649
FXYD domain containing ion transport regulator 1 (phospholemman)	0.862 (0.561 to 1.092)	0.561
FXYD domain containing ion transport regulator 3	0.964 (0.934 to 0.995)	0.452
FYN binding protein (FYB-120/130)	1.222 (0.741 to 2.007)	0.558
FYN oncogene related to SRC, FGR, YES	1.237 (0.534 to 3.218)	0.605
fyn-related kinase	0.911 (0.626 to 1.11)	0.666
G antigen 1	1.074 (0.846 to 1.46)	0.6
G antigen 2	1.122 (0.641 to 1.636)	0.635
G antigen 3	0.665 (0.331 to 1.338)	0.664
G antigen 4	1.24 (1.041 to 1.374)	0.134
G antigen, family B, 1 (prostate associated)	1.176 (0.865 to 2.08)	0.467
G protein pathway suppressor 1	1.176 (1.109 to 1.248)	0.222
G protein pathway suppressor 2	0.995 (0.44 to 2.302)	0.992
G protein-coupled receptor 1	0.909 (0.354 to 1.954)	0.76
G protein-coupled receptor 12	1.057 (1.004 to 1.112)	0.475
G protein-coupled receptor 15	1.015 (0.851 to 1.145)	0.886
G protein-coupled receptor 17	1.098 (0.972 to 1.363)	0.48
G protein-coupled receptor 19	0.998 (0.994 to 1.003)	0.751
G protein-coupled receptor 20	0.536 (0.353 to 0.804)	0.0697
G protein-coupled receptor 21	0.714 (0.444 to 1.053)	0.313
G protein-coupled receptor 22	1.599 (0.99 to 3.273)	0.129
G protein-coupled receptor 23	0.972 (0.88 to 1.166)	0.785
G protein-coupled receptor 24	1.093 (1.082 to 1.104)	0.0726
G protein-coupled receptor 25	0.916 (0.474 to 1.9)	0.847

G protein-coupled receptor 3	1.099 (0.988 to 1.223)	0.539
G protein-coupled receptor 30	1.489 (1.315 to 1.687)	0.193
G protein-coupled receptor 31	0.986 (0.946 to 1.028)	0.798
G protein-coupled receptor 32	1.05 (0.825 to 1.361)	0.769
G protein-coupled receptor 35	1.634 (0.754 to 2.378)	0.0748
G protein-coupled receptor 37 (endothelin receptor type B-like)	0.545 (0.453 to 0.657)	0.19
G protein-coupled receptor 38	0.976 (0.795 to 1.11)	0.758
G protein-coupled receptor 4	1.035 (0.858 to 1.294)	0.741
G protein-coupled receptor 40	0.805 (0.298 to 1.439)	0.581
G protein-coupled receptor 41	1.993 (1.455 to 3.04)	0.088
G protein-coupled receptor 43	1.094 (0.908 to 1.319)	0.713
G protein-coupled receptor 6	0.89	no replicates
G protein-coupled receptor 65	1.27 (0.831 to 1.62)	0.378
G protein-coupled receptor 68	0.999 (0.823 to 1.249)	0.993
G protein-coupled receptor 7	2.293 (1.875 to 2.985)	0.0265
G protein-coupled receptor 8	0.773	no replicates
G protein-coupled receptor kinase 2-like (Drosophila)	6.175 (0.185 to 46.03)	0.165
G protein-coupled receptor kinase 6	1	no replicates
G1 to S phase transition 1	0.768 (0.407 to 1.167)	0.334
GA binding protein transcription factor, alpha subunit 60kDa	1.099 (0.88 to 1.283)	0.494
GA binding protein transcription factor, beta subunit 2, 47kDa	1.007 (0.631 to 1.637)	0.979
galactokinase 1	0.944 (0.506 to 1.392)	0.762
galactokinase 2	1.335 (0.819 to 2.178)	0.66
galactosamine (N-acetyl)-6-sulfate sulfatase (Morquio syndrome, mucopolysaccharidosis type IVA)	1.219 (0.949 to 1.566)	0.574
galactose-1-phosphate uridylyltransferase	1.233 (1.074 to 1.417)	0.371
galactosidase, alpha	1.004 (0.793 to 1.27)	0.991
galactosylceramidase (Krabbe disease)	1.088 (0.821 to 1.324)	0.369
galanin receptor 3	1.06 (1.047 to 1.073)	0.135
gamma-aminobutyric acid (GABA) A receptor, alpha 1	1.282 (1.038 to 1.584)	0.449
gamma-aminobutyric acid (GABA) A receptor, alpha 2	1.093 (0.835 to 1.733)	0.563
gamma-aminobutyric acid (GABA) A receptor, alpha 3	1.072 (0.894 to 1.285)	0.576
gamma-aminobutyric acid (GABA) A receptor, alpha 4	0.998 (0.88 to 1.132)	0.992
gamma-aminobutyric acid (GABA) A receptor, alpha 5	0.771 (0.374 to 1.348)	0.297
gamma-aminobutyric acid (GABA) A receptor, alpha 6	1.226 (1.009 to 1.49)	0.486
gamma-aminobutyric acid (GABA) A receptor, beta 2	0.792 (0.558 to 1.053)	0.199
gamma-aminobutyric acid (GABA) A receptor, beta 3	1.74 (1.474 to 1.981)	0.00286
gamma-aminobutyric acid (GABA) A receptor, delta	0.918 (0.73 to 1.156)	0.774
gamma-aminobutyric acid (GABA) B receptor, 1	1.208 (0.82 to 2.174)	0.303
gamma-aminobutyric acid (GABA) receptor, rho 1	1.322 (0.957 to 1.951)	0.312
gamma-aminobutyric acid (GABA) receptor, rho 2	1.067 (0.755 to 1.294)	0.626
gamma-glutamyl carboxylase	0.614 (0.572 to 0.658)	0.0903
gamma-glutamyltransferase 1	1.087 (0.793 to 1.528)	0.629
gamma-glutamyltransferase-like activity 1	1.009 (0.836 to 1.247)	0.905
gap junction protein, alpha 1, 43kDa (connexin 43)	1.099 (0.972 to 1.243)	0.581
gap junction protein, alpha 4, 37kDa (connexin 37)	1.952 (1.94 to 1.964)	0.00575
gap junction protein, alpha 5, 40kDa (connexin 40)	1.015 (0.616 to 1.485)	0.933
gap junction protein, alpha 8, 50kDa (connexin 50)	1.048 (0.764 to 1.258)	0.794
gap junction protein, beta 1, 32kDa (connexin 32, Charcot-Marie-Tooth neuropathy, X-linked)	0.776 (0.554 to 0.955)	0.272
Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog	0.501 (0.128 to 1.918)	0.471
gastric inhibitory polypeptide	1.115 (1.01 to 1.203)	0.0758
gastric inhibitory polypeptide receptor	1.445 (1.345 to 1.552)	0.122
gastrin	1.224 (0.909 to 1.534)	0.322
gastrulation brain homeo box 2	1.114 (0.629 to 1.646)	0.607
GATA binding protein 1 (globin transcription factor 1)	1.048 (0.875 to 1.18)	0.661
GATA binding protein 2	1.362 (0.914 to 1.831)	0.274
GATA binding protein 3	1.11 (0.759 to 1.31)	0.474
GATA binding protein 6	0.663 (0.284 to 1.326)	0.459
GCN5 general control of amino-acid synthesis 5-like 1 (yeast)	0.468 (0.259 to 0.649)	0.125
GDNF family receptor alpha 1	1.202 (0.521 to 2.969)	0.64
GDNF family receptor alpha 2	1.519 (0.801 to 3.6)	0.183
GDNF family receptor alpha 3	1.154 (0.722 to 2.863)	0.596
GDP dissociation inhibitor 1	1.289 (0.999 to 1.662)	0.226
GDP dissociation inhibitor 2	1.371 (1 to 1.878)	0.5
GDP-mannose 4,6-dehydratase	1.047 (0.894 to 1.16)	0.496
gelsolin (amyloidosis, Finnish type)	1.477 (0.729 to 2.609)	0.406
general transcription factor II, i	0.613 (0.0422 to 6.892)	0.688
general transcription factor IIA, 2, 12kDa	0.969 (0.534 to 1.56)	0.898
general transcription factor IIE, polypeptide 1, alpha 56kDa	1.061 (0.565 to 1.435)	0.798

general transcription factor IIE, polypeptide 2, beta 34kDa	1.177 (1.116 to 1.241)	0.2
general transcription factor IIF, polypeptide 1, 74kDa	0.746 (0.604 to 0.921)	0.397
general transcription factor IIF, polypeptide 2, 30kDa	0.95 (0.613 to 1.794)	0.808
general transcription factor IIH, polypeptide 1, 62kDa	1.312 (1.012 to 2.166)	0.392
general transcription factor IIH, polypeptide 3, 34kDa	0.91 (0.822 to 1.015)	0.262
general transcription factor IIH, polypeptide 4, 52kDa	1.747 (0.765 to 3.99)	0.622
general transcription factor IIIA	0.745 (0.491 to 0.999)	0.147
general transcription factor IIIC, polypeptide 1, alpha 220kDa	0.927 (0.839 to 1.039)	0.19
GLE1 RNA export mediator-like (yeast)	1.11 (0.693 to 1.996)	0.592
glia maturation factor, beta	0.95 (0.902 to 1.008)	0.147
glial cells missing homolog 1 (Drosophila)	1.417 (1.006 to 1.982)	0.0897
glial fibrillary acidic protein	1.016	no replicates
GLI-Kruppel family member GLI2	1.085 (1.065 to 1.106)	0.145
GLI-Kruppel family member GLI3 (Greig cephalopolysyndactyly syndrome)	0.538 (0.313 to 0.923)	0.456
GLI-Kruppel family member HKR3	1.081 (0.643 to 1.7)	0.726
glioblastoma amplified sequence	1.163 (1.046 to 1.352)	0.0681
glioma-associated oncogene homolog (zinc finger protein)	1.126 (1 to 1.209)	0.186
glucagon receptor	0.819 (0.636 to 1.054)	0.573
glucagon-like peptide 1 receptor	0.959 (0.64 to 1.436)	0.934
glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme, Andersen disease, glycogen storage disease type IV)	1.023 (0.923 to 1.103)	0.712
glucocorticoid receptor DNA binding factor 1	1.979 (1.704 to 2.298)	0.137
glucokinase (hexokinase 4) regulatory protein	0.998 (0.74 to 1.282)	0.983
glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	1.128 (0.771 to 1.784)	0.471
glucosaminyl (N-acetyl) transferase 1, core 2 (beta-1,6-N-acetylglucosaminyltransferase)	1.661 (1.485 to 1.858)	0.138
glucose phosphate isomerase	1.622 (1.051 to 2.503)	0.465
glucose regulated protein, 58kDa	0.849 (0.541 to 1.069)	0.543
glucose-6-phosphatase, catalytic (glycogen storage disease type I, von Gierke disease)	0.611 (0.365 to 1.023)	0.514
glucose-6-phosphatase, transport (glucose-6-phosphate) protein 1	1.144 (0.939 to 1.611)	0.516
glucose-6-phosphate dehydrogenase	1.041 (0.684 to 1.317)	0.866
glucosidase, alpha; acid (Pompe disease, glycogen storage disease type II)	1.1 (0.956 to 1.267)	0.62
glucosidase, beta; acid (includes glucosylceramidase)	1.258 (1.258 to 1.259)	0.000369
glucuronidase, beta	1.218 (0.758 to 1.989)	0.552
glutamate dehydrogenase 1	1.062 (0.978 to 1.153)	0.599
glutamate receptor, ionotrophic, AMPA 3	1.35 (0.901 to 1.889)	0.187
glutamate receptor, ionotrophic, AMPA 4	1.841 (1.712 to 1.979)	0.0753
glutamate receptor, ionotropic, AMPA 1	0.958 (0.827 to 1.09)	0.487
glutamate receptor, ionotropic, delta 2	0.868 (0.829 to 0.908)	0.197
glutamate receptor, ionotropic, kainate 1	0.744 (0.691 to 0.801)	0.156
glutamate receptor, ionotropic, kainate 3	1.98 (1.807 to 2.17)	0.0847
glutamate receptor, ionotropic, N-methyl D-aspartate 1	0.706 (0.513 to 0.957)	0.0368
glutamate receptor, ionotropic, N-methyl D-aspartate 2A	1.743 (1.537 to 1.978)	0.142
glutamate receptor, ionotropic, N-methyl D-aspartate 2C	1.077 (0.79 to 1.488)	0.683
glutamate receptor, ionotropic, N-methyl D-aspartate 2D	0.994 (0.888 to 1.113)	0.968
glutamate receptor, metabotropic 2	1.182 (0.652 to 1.889)	0.531
glutamate receptor, metabotropic 3	1.978 (1.542 to 2.536)	0.223
glutamate receptor, metabotropic 4	1.568 (0.775 to 3.17)	0.638
glutamate receptor, metabotropic 5	1.47 (0.534 to 5.849)	0.497
glutamate receptor, metabotropic 6	1.09 (0.671 to 1.401)	0.57
glutamate receptor, metabotropic 7	0.768 (0.735 to 0.838)	0.026
glutamate receptor, metabotropic 8	0.856 (0.71 to 1.033)	0.56
glutamate-ammonia ligase (glutamine synthase)	1.158 (0.679 to 1.993)	0.498
glutamate-cysteine ligase, catalytic subunit	1.239 (1.107 to 1.386)	0.308
glutamate-cysteine ligase, modifier subunit	1.192 (0.988 to 1.729)	0.264
glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)	1.071 (0.687 to 1.477)	0.792
glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)	1.159 (1.066 to 1.261)	0.33
glutamic-pyruvate transaminase (alanine aminotransferase)	1.123 (1.002 to 1.258)	0.494
glutamine-fructose-6-phosphate transaminase 1	1.851 (0.909 to 3.773)	0.546
glutaminyl-tRNA synthetase	0.929 (0.818 to 1.054)	0.663
glutamyl aminopeptidase (aminopeptidase A)	0.952 (0.637 to 1.372)	0.755
glutamyl-prolyl-tRNA synthetase	0.967 (0.775 to 1.2)	0.731
glutaredoxin (thioltransferase)	1.585 (1.102 to 2.28)	0.425
glutaryl-Coenzyme A dehydrogenase	0.722	no replicates
glutathione peroxidase 3 (plasma)	0.89 (0.221 to 3.581)	0.947
glutathione peroxidase 4 (phospholipid hydroperoxidase)	0.736 (0.332 to 1.34)	0.537

glutathione peroxidase 5 (epididymal androgen-related protein)	1.355 (0.607 to 3.06)	0.426
glutathione S-transferase A1	1.273 (0.971 to 1.671)	0.537
glutathione S-transferase A4	2.081 (1.041 to 5.88)	0.3
glutathione S-transferase M2 (muscle)	1.093 (0.981 to 1.216)	0.561
glutathione S-transferase M3 (brain)	0.981 (0.592 to 1.348)	0.921
glutathione S-transferase M4	0.711 (0.467 to 1.231)	0.357
glutathione S-transferase M5	1.238 (0.763 to 2.659)	0.636
glutathione S-transferase pi	1.007 (0.763 to 2.046)	0.971
glutathione S-transferase theta 2	0.995	no replicates
glutathione synthetase	1.252 (1.053 to 1.488)	0.419
glutathione transferase zeta 1 (maleylacetoacetate isomerase)	0.938 (0.413 to 1.692)	0.848
glyceraldehyde-3-phosphate dehydrogenase	1.03 (0.492 to 1.846)	0.909
glycerol kinase	1.267 (0.321 to 5.01)	0.891
glycerol-3-phosphate dehydrogenase 1 (soluble)	0.718 (0.423 to 1.174)	0.379
glycerol-3-phosphate dehydrogenase 2 (mitochondrial)	1.015 (0.908 to 1.111)	0.823
glycine amidinotransferase (L-arginine:glycine amidinotransferase)	1.121 (0.774 to 1.479)	0.405
glycine cleavage system protein H (aminomethyl carrier)	0.937 (0.825 to 1.063)	0.697
glycine dehydrogenase (decarboxylating; glycine decarboxylase, glycine cleavage system protein P)	0.8	no replicates
glycine receptor, alpha 1 (startle disease/hyperekplexia, stiff man syndrome)	0.633 (0.419 to 0.826)	0.16
glycine receptor, alpha 2	0.84 (0.677 to 1.167)	0.405
glycogen synthase 1 (muscle)	0.701 (0.371 to 1.338)	0.278
glycogen synthase kinase 3 beta	0.877 (0.656 to 1.194)	0.374
glycogenin	1.542 (0.72 to 4.914)	0.264
glycophorin A (includes MN blood group)	0.584 (0.343 to 0.993)	0.496
glycophorin B (includes Ss blood group)	0.803 (0.654 to 1.147)	0.345
glycophorin C (Gerbich blood group)	1.456 (0.877 to 2.42)	0.594
glycoprotein 2 (zymogen granule membrane)	1.391 (1.343 to 1.44)	0.0671
glycoprotein A33 (transmembrane)	1.09 (0.863 to 1.378)	0.775
glycoprotein Ib (platelet), alpha polypeptide	1.014 (0.902 to 1.139)	0.925
glycoprotein Ib (platelet), beta polypeptide	0.91 (0.583 to 1.422)	0.868
glycoprotein IX (platelet)	1.014 (0.897 to 1.147)	0.927
glycoprotein V (platelet)	0.885	no replicates
glyoxalase I	1.104 (0.874 to 1.396)	0.745
glypican 1	0.853 (0.627 to 1.419)	0.442
glypican 3	1.044 (0.609 to 1.711)	0.807
glypican 4	1.023 (0.866 to 1.208)	0.914
GM2 ganglioside activator protein	1.132 (0.971 to 1.356)	0.0806
golgi autoantigen, golgin subfamily a, 1	1.032 (0.742 to 1.4)	0.825
golgi autoantigen, golgin subfamily a, 2	1.136 (0.638 to 1.767)	0.592
golgi autoantigen, golgin subfamily a, 3	1.07 (0.931 to 1.231)	0.712
golgi autoantigen, golgin subfamily a, 4	1.019 (0.508 to 1.73)	0.947
golgi-specific brefeldin A resistance factor 1	0.956 (0.719 to 1.504)	0.862
gonadotropin-releasing hormone 1 (leutinizing-releasing hormone)	1.317 (0.709 to 2.447)	0.734
gonadotropin-releasing hormone 2	1.08 (1.066 to 1.093)	0.1
gonadotropin-releasing hormone receptor	0.837 (0.67 to 1.11)	0.353
goosecoid-like	1.169 (0.765 to 2.354)	0.702
GPAA1P anchor attachment protein 1 homolog (yeast)	0.738 (0.492 to 1.108)	0.591
GPI anchored molecule like protein	1.791 (1.584 to 1.957)	0.0116
granulin	1.317 (0.539 to 2.401)	0.371
granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	0.868 (0.668 to 1.082)	0.222
granzyme K (serine protease, granzyme 3; tryptase II)	0.991 (0.919 to 1.058)	0.843
granzyme M (lymphocyte met-ase 1)	1.235 (1.063 to 1.434)	0.393
GRB2-associated binding protein 1	2.199 (1.682 to 2.874)	0.209
G-rich RNA sequence binding factor 1	1.207 (0.907 to 1.593)	0.367
growth arrest-specific 2	1.014 (0.631 to 1.555)	0.948
growth arrest-specific 6	1.576 (1.526 to 1.626)	0.0443
growth arrest-specific 7	1.048 (0.953 to 1.112)	0.434
growth differentiation factor 10	1.118 (0.561 to 1.759)	0.588
growth differentiation factor 11	1.273 (1.169 to 1.387)	0.217
growth differentiation factor 5 (cartilage-derived morphogenetic protein-1)	1.016 (0.861 to 1.362)	0.926
growth differentiation factor 8	1.113 (0.524 to 1.948)	0.649
growth differentiation factor 9	0.86 (0.521 to 1.127)	0.608
growth factor independent 1	1.135 (0.896 to 1.454)	0.365
growth factor independent 1B (potential regulator of CDKN1A, translocated in CML)	1.171 (1.076 to 1.273)	0.311
growth factor receptor-bound protein 14	0.995 (0.899 to 1.101)	0.966
growth factor receptor-bound protein 7	1.564 (1.277 to 1.757)	0.0479

growth hormone 1	1.107 (0.85 to 1.327)	0.299
growth hormone receptor	1.394 (1.225 to 1.586)	0.236
growth hormone releasing hormone receptor	1.919 (1.411 to 2.61)	0.281
growth hormone secretagogue receptor	1.096 (0.741 to 1.807)	0.638
GTP binding protein overexpressed in skeletal muscle	0.81 (0.577 to 1.137)	0.646
GTP cyclohydrolase 1 (dopa-responsive dystonia)	0.783 (0.673 to 0.91)	0.35
GTP cyclohydrolase I feedback regulatory protein	0.888 (0.792 to 0.997)	0.491
guanidinoacetate N-methyltransferase	1.061 (0.911 to 1.2)	0.542
guanine nucleotide binding protein (G protein) alpha 12	1.777 (1.092 to 2.893)	0.447
guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	0.901 (0.624 to 1.221)	0.649
guanine nucleotide binding protein (G protein), alpha 15 (Gq class)	1.049 (0.511 to 2.002)	0.876
guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type	1.167 (0.629 to 1.7)	0.669
guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	0.902 (0.647 to 1.279)	0.654
guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	0.553 (0.354 to 0.713)	0.118
guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3	0.943 (0.637 to 1.602)	0.85
guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 1	1.444	no replicates
guanine nucleotide binding protein (G protein), alpha z polypeptide	1.589 (1.149 to 2.395)	0.166
guanine nucleotide binding protein (G protein), beta polypeptide 3	1.219 (0.78 to 2.018)	0.353
guanine nucleotide binding protein (G protein), gamma 10	0.968 (0.792 to 1.116)	0.782
guanine nucleotide binding protein (G protein), gamma 11	0.999 (0.719 to 1.348)	0.994
guanine nucleotide binding protein (G protein), gamma 4	0.9 (0.766 to 1.136)	0.469
guanine nucleotide binding protein (G protein), gamma 5	0.663 (0.163 to 1.231)	0.321
guanine nucleotide binding protein (G protein), gamma 7	0.847 (0.823 to 0.872)	0.109
guanine nucleotide binding protein (G protein), q polypeptide	0.832 (0.687 to 1.008)	0.513
guanine nucleotide binding protein-like 1	1.072 (1.007 to 1.142)	0.467
guanine nucleotide-releasing factor 2 (specific for crk proto-oncogene)	0.981 (0.819 to 1.167)	0.841
guanylate binding protein 1, interferon-inducible, 67kDa	1.379 (1.216 to 1.563)	0.237
guanylate binding protein 2, interferon-inducible	1.352 (0.737 to 2.431)	0.474
guanylate cyclase 1, soluble, alpha 3	1.048 (0.991 to 1.108)	0.557
guanylate cyclase activator 2A (guanylin)	1.346 (1.159 to 1.563)	0.297
guanylate cyclase activator 2B (uroguanylin)	1.013 (0.806 to 1.258)	0.905
guanylate kinase 1	1.54 (1.142 to 2.076)	0.385
H factor (complement)-like 3	1.159 (0.833 to 1.684)	0.351
H factor 1 (complement)	0.891 (0.638 to 1.096)	0.563
H1 histone family, member 0	0.711 (0.317 to 1.109)	0.191
H2A histone family, member X	1.304 (1.133 to 1.501)	0.31
H2A histone family, member Z	1.622 (1.225 to 1.974)	0.0783
H3 histone, family 3A	0.862 (0.653 to 1.07)	0.255
H3 histone, family 3B (H3.3B)	1.371 (1.011 to 1.858)	0.489
hairy and enhancer of split 1, (Drosophila)	1.324 (0.829 to 3.498)	0.342
harakiri, BCL2 interacting protein (contains only BH3 domain)	0.383 (0.31 to 0.472)	0.138
heat shock 10kDa protein 1 (chaperonin 10)	1.292 (1.209 to 1.379)	0.16
heat shock 27kDa protein 2	0.952 (0.612 to 1.338)	0.743
heat shock 70kDa protein 1B	0.905 (0.717 to 1.143)	0.743
heat shock 70kDa protein 1-like	0.973 (0.731 to 2.111)	0.896
heat shock 70kDa protein 6 (HSP70B')	1.036 (0.834 to 1.148)	0.567
heat shock 70kDa protein 9B (mortalin-2)	1.034 (0.637 to 2.017)	0.903
heat shock 90kDa protein 1, beta	1.116 (0.898 to 1.915)	0.589
heat shock factor binding protein 1	1.753 (1.24 to 2.479)	0.352
heat shock transcription factor 2	1.335 (0.711 to 2.46)	0.338
heat shock transcription factor 4	0.814 (0.285 to 1.904)	0.553
hematopoietic cell-specific Lyn substrate 1	1.585 (1.304 to 2.479)	0.0164
hematopoietic protein 1	0.838 (0.562 to 1.074)	0.474
hematopoietically expressed homeobox	1.121 (0.853 to 1.493)	0.331
heme oxygenase (decycling) 2	1.009 (0.72 to 1.336)	0.956
hemochromatosis	1.319 (1.313 to 1.324)	0.00924
hemoglobin, alpha 1	0.692 (0.477 to 1.006)	0.505
hemoglobin, alpha 2	0.928 (0.839 to 1.027)	0.597
hemoglobin, beta	0.663 (0.295 to 1.126)	0.168
hemoglobin, delta	1.399 (1.09 to 2.46)	0.174
hemoglobin, epsilon 1	0.952 (0.663 to 1.276)	0.742
hemoglobin, gamma A	1.145 (0.931 to 1.273)	0.162
hemoglobin, theta 1	1.244 (1.047 to 1.612)	0.239
hemoglobin, zeta	1.658 (1.092 to 2.518)	0.44
hepsin (transmembrane protease, serine 1)	0.754 (0.561 to 0.946)	0.21

Hermansky-Pudlak syndrome 1	0.985 (0.773 to 1.174)	0.913
heterogeneous nuclear ribonucleoprotein A/B	0.789 (0.455 to 1.031)	0.301
heterogeneous nuclear ribonucleoprotein A1	1.187 (0.994 to 1.609)	0.223
heterogeneous nuclear ribonucleoprotein A2/B1	1.968 (1.381 to 2.805)	0.307
heterogeneous nuclear ribonucleoprotein C (C1/C2)	1.024 (0.906 to 1.256)	0.837
heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)	1.01 (0.82 to 1.382)	0.919
heterogeneous nuclear ribonucleoprotein F	1.194 (1.029 to 1.408)	0.0733
heterogeneous nuclear ribonucleoprotein K	0.985 (0.764 to 1.244)	0.868
heterogeneous nuclear ribonucleoprotein L	0.718 (0.427 to 1.208)	0.639
heterogeneous nuclear ribonucleoprotein M	0.836 (0.662 to 1.202)	0.433
heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A)	1.564 (0.666 to 9.547)	0.413
hexokinase 1	1.225 (1.15 to 1.304)	0.191
hexokinase 3 (white cell)	1.339 (1.282 to 1.398)	0.0935
hexosaminidase A (alpha polypeptide)	1.51 (1.146 to 2.497)	0.243
hexosaminidase B (beta polypeptide)	1.134 (1.044 to 1.232)	0.369
high density lipoprotein binding protein (vigilin)	0.75 (0.539 to 0.931)	0.228
high-mobility group box 1	1.112 (0.818 to 1.513)	0.788
high-mobility group box 3	1.775 (1.509 to 1.974)	0.02
high-mobility group nucleosomal binding domain 2	1.085 (0.426 to 2.168)	0.788
high-mobility group nucleosome binding domain 1	1.025 (0.871 to 1.231)	0.807
hippocalcin	1.209 (1.127 to 1.297)	0.226
hippocalcin-like 1	1.046 (0.374 to 2.817)	0.901
HIR histone cell cycle regulation defective homolog A (S. cerevisiae)	1.904 (0.797 to 4.551)	0.595
HIRA interacting protein 3	1.11 (0.871 to 1.416)	0.742
histamine N-methyltransferase	2.098 (1.423 to 2.741)	0.0649
histamine receptor H1	1.068 (0.756 to 2.203)	0.753
histatin 1	0.75 (0.615 to 0.847)	0.0256
histatin 3	1.434 (0.962 to 3.119)	0.451
histidine ammonia-lyase	0.964 (0.742 to 1.443)	0.823
histidine rich calcium binding protein	1.04 (0.71 to 1.497)	0.814
histidine-rich glycoprotein	0.961 (0.504 to 1.43)	0.915
histidyl-tRNA synthetase	0.828 (0.695 to 0.988)	0.205
histone 1, H1a	1.117 (0.986 to 1.266)	0.538
histone 1, H1c	1.414 (1.108 to 1.861)	0.0776
histone 1, H1d	1.099 (0.978 to 1.235)	0.566
histone 1, H1e	1.099 (0.665 to 2.544)	0.71
histone 1, H2ac	0.866 (0.636 to 1.18)	0.723
histone 1, H2bl	0.9 (0.802 to 0.999)	0.238
histone 1, H2bn	1.015 (0.598 to 1.869)	0.968
histone 1, H3h	1.463 (1.078 to 1.984)	0.43
histone 1, H4a	1.097 (1.065 to 1.129)	0.195
histone 1, H4c	1.006 (0.951 to 1.056)	0.859
histone 1, H4d	0.936 (0.737 to 1.12)	0.546
histone 1, H4e	0.67 (0.26 to 1.261)	0.319
histone 1, H4f	1.221 (1.06 to 1.578)	0.261
histone 1, H4h	1.141 (0.839 to 1.592)	0.552
histone 2, H2be	1.124 (0.533 to 2.186)	0.64
histone acetyltransferase 1	0.918 (0.87 to 0.968)	0.356
histone deacetylase 2	1.574 (1.275 to 2.03)	0.0178
HIV-1 Rev binding protein	1.154 (0.822 to 1.62)	0.746
HLA-B associated transcript 1	1.73 (0.976 to 4.447)	0.217
HLA-B associated transcript 3	1.1 (0.752 to 2.268)	0.816
HLA-G histocompatibility antigen, class I, G	0.876 (0.576 to 1.213)	0.466
HMT1 hnRNP methyltransferase-like 1 (S. cerevisiae)	0.915 (0.662 to 1.125)	0.49
holocarboxylase synthetase (biotin-[proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)] ligase)	1.118 (1.027 to 1.216)	0.412
holocytochrome c synthase (cytochrome c heme-lyase)	1.356 (1.257 to 1.462)	0.154
homeo box (expressed in ES cells) 1	1.281 (0.912 to 1.863)	0.294
homeo box A11	1.325 (1.064 to 1.641)	0.0246
homeo box A13	0.941 (0.681 to 1.46)	0.674
homeo box A4	0.941 (0.599 to 1.785)	0.812
homeo box A7	1.701 (1.171 to 2.783)	0.0297
homeo box A9	1.13 (0.84 to 1.316)	0.311
homeo box B1	0.988 (0.747 to 1.77)	0.956
homeo box B2	1.267 (1.029 to 1.833)	0.0869
homeo box B3	1.137 (0.988 to 1.259)	0.219
homeo box B5	1.104 (0.743 to 1.614)	0.606
homeo box B7	1.548 (1.404 to 1.706)	0.14

homeo box C6	0.904 (0.56 to 1.264)	0.606
homeo box D10	1.06 (0.947 to 1.43)	0.488
homeo box HB9	1.292 (1.09 to 1.531)	0.373
homogentisate 1,2-dioxygenase (homogentisate oxidase)	1.262 (1.025 to 1.554)	0.464
host cell factor C1 (VP16-accessory protein)	0.734 (0.464 to 1.014)	0.159
human immunodeficiency virus type I enhancer binding protein 1	0.804 (0.403 to 1.208)	0.346
human T-cell leukemia virus enhancer factor	1.267 (1.252 to 1.282)	0.0311
huntingtin interacting protein 1	0.775 (0.362 to 1.479)	0.598
huntingtin interacting protein 2	1.493	no replicates
HUS1 checkpoint homolog (S. pombe)	1.736 (0.843 to 5.902)	0.464
hyaluronan synthase 1	0.889 (0.618 to 1.228)	0.615
hyaluronan synthase 2	1.194 (1.07 to 1.334)	0.353
hyaluronoglucosaminidase 1	1.01 (0.842 to 1.295)	0.944
hyaluronoglucosaminidase 3	0.71 (0.556 to 1.088)	0.251
hydroxyacyl glutathione hydrolase	1.037 (0.725 to 1.296)	0.858
hydroxyacyl-Coenzyme A dehydrogenase, type II	0.848 (0.648 to 1.108)	0.648
hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit	1.007 (0.929 to 1.105)	0.864
hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit	1.203 (0.976 to 1.556)	0.31
hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	0.831 (0.39 to 1.433)	0.682
hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2	1.258 (0.756 to 2.724)	0.347
hydroxymethylbilane synthase	1.048 (0.907 to 1.305)	0.587
hydroxyprostaglandin dehydrogenase 15-(NAD)	1.127 (1.072 to 1.185)	0.252
hydroxysteroid (11-beta) dehydrogenase 1	1.095 (0.425 to 1.835)	0.758
hydroxysteroid (11-beta) dehydrogenase 2	0.836 (0.43 to 1.625)	0.832
hydroxysteroid (17-beta) dehydrogenase 1	1.093 (0.629 to 2.052)	0.755
hydroxysteroid (17-beta) dehydrogenase 2	0.933 (0.841 to 1.045)	0.384
hydroxysteroid (17-beta) dehydrogenase 3	1.492 (0.669 to 3.564)	0.355
hydroxysteroid (17-beta) dehydrogenase 4	1.165 (1.162 to 1.168)	0.0114
hypocretin (orexin) receptor 1	0.825 (0.648 to 1)	0.129
hypocretin (orexin) receptor 2	1.056 (0.784 to 1.452)	0.651
hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)	1.188 (0.89 to 1.586)	0.658
I factor (complement)	1.525 (1.474 to 1.577)	0.0508
iduronate 2-sulfatase (Hunter syndrome)	1.035 (0.516 to 2.098)	0.912
iduronidase, alpha-L-	1.082 (0.466 to 1.965)	0.838
IK cytokine, down-regulator of HLA II	1.022 (0.595 to 1.373)	0.943
IL2-inducible T-cell kinase	1.207 (1.114 to 1.408)	0.134
immature colon carcinoma transcript 1	1.358 (0.892 to 2.067)	0.6
immunoglobulin (CD79A) binding protein 1	1.608	no replicates
immunoglobulin mu binding protein 2	0.976 (0.493 to 1.24)	0.894
immunoglobulin superfamily containing leucine-rich repeat	0.964 (0.768 to 1.151)	0.786
immunoglobulin superfamily, member 1	1.586 (1.074 to 2.738)	0.243
immunoglobulin superfamily, member 3	1.182 (0.553 to 2.654)	0.648
IMP (inosine monophosphate) dehydrogenase 2	0.81 (0.412 to 1.168)	0.333
inactivation escape 1	1.062 (0.846 to 1.203)	0.649
indoleamine-pyrrole 2,3 dioxygenase	0.826 (0.502 to 1.36)	0.767
inhibin, beta C	0.93 (0.774 to 1.173)	0.487
inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	0.991 (0.818 to 1.278)	0.936
inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	1.029 (0.945 to 1.121)	0.795
inhibitor of growth family, member 1-like	1 (0.618 to 1.493)	0.999
inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	0.511 (0.406 to 0.642)	0.209
inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	0.811 (0.491 to 1.081)	0.491
inositol 1,4,5-triphosphate receptor, type 2	0.913 (0.793 to 1.187)	0.404
inositol 1,4,5-triphosphate receptor, type 3	1.239 (0.773 to 1.788)	0.375
inositol 1,4,5-trisphosphate 3-kinase A	0.922 (0.634 to 1.385)	0.631
inositol 1,4,5-trisphosphate 3-kinase B	1.091 (0.979 to 1.216)	0.569
inositol polyphosphate phosphatase-like 1	1.046 (1.009 to 1.085)	0.434
inositol polyphosphate-1-phosphatase	0.948 (0.326 to 1.409)	0.851
inositol polyphosphate-4-phosphatase, type I, 107kDa	1.032 (0.959 to 1.131)	0.459
inositol polyphosphate-4-phosphatase, type II, 105kDa	1.175 (0.328 to 2.244)	0.679
inositol polyphosphate-5-phosphatase, 145kDa	1.333 (0.829 to 3.411)	0.304
inositol polyphosphate-5-phosphatase, 40kDa	1.369 (0.93 to 2.739)	0.461
inositol(myo)-1(or 4)-monophosphatase 1	1.039 (0.68 to 1.747)	0.86
insulin	1.446 (1.236 to 1.691)	0.256
insulin induced gene 1	1.089 (0.898 to 1.301)	0.347

insulin promoter factor 1, homeodomain transcription factor	1.186 (1.095 to 1.283)	0.277
insulin receptor substrate 1	0.715 (0.534 to 0.946)	0.123
insulin receptor substrate 2	0.853 (0.759 to 0.959)	0.403
insulin receptor substrate 4	0.809 (0.322 to 1.425)	0.446
insulin-like 3 (Leydig cell)	0.73 (0.332 to 1.838)	0.451
insulin-like 4 (placenta)	2.957 (1.955 to 4.473)	0.232
insulin-like growth factor 2 (somatomedin A)	1.188 (0.842 to 1.618)	0.254
insulin-like growth factor binding protein 1	1.184 (0.809 to 2.538)	0.56
insulin-like growth factor binding protein 3	1.795 (1.013 to 3.18)	0.493
insulin-like growth factor binding protein 7	1.191 (1.008 to 1.409)	0.486
insulin-like growth factor binding protein, acid labile subunit	0.944 (0.766 to 1.203)	0.546
insulinoma-associated 1	0.913 (0.776 to 1.073)	0.673
integral membrane protein 1	0.472 (0.0606 to 2.208)	0.555
integrin beta 4 binding protein	2.356 (1.882 to 2.647)	0.0167
integrin, alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41B)	1.278 (0.77 to 2.238)	0.292
integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)	1.018 (0.861 to 1.221)	0.875
integrin, alpha 7	1.428 (0.569 to 2.805)	0.41
integrin, alpha D	1.048 (0.917 to 1.351)	0.622
integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	0.491 (0.0602 to 1.407)	0.568
integrin, alpha X (antigen CD11C (p150), alpha polypeptide)	1.911 (1.263 to 3.776)	0.2
integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)	1.052 (0.805 to 1.226)	0.741
integrin, beta 4	0.574 (0.454 to 0.716)	0.052
integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II)	1.379 (0.937 to 1.731)	0.24
inter-alpha (globulin) inhibitor, H1 polypeptide	0.971 (0.56 to 1.271)	0.849
intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	1.935 (0.982 to 3.814)	0.509
intercellular adhesion molecule 3	1.035 (0.356 to 1.775)	0.912
intercellular adhesion molecule 4, Landsteiner-Wiener blood group	0.92 (0.68 to 1.203)	0.552
intercellular adhesion molecule 5, telencephalin	0.645 (0.502 to 0.829)	0.331
interferon regulatory factor 3	0.782 (0.378 to 1.202)	0.295
interferon regulatory factor 7	1.14 (0.929 to 1.623)	0.32
interferon stimulated gene 20kDa	0.852 (0.627 to 1.363)	0.425
interferon, alpha 10	1.432 (0.52 to 9.881)	0.624
interferon, alpha 14	1.273 (0.964 to 2.079)	0.251
interferon, alpha 16	1.559 (1.072 to 2.749)	0.264
interferon, alpha 21	1.117 (0.941 to 1.267)	0.338
interferon, alpha 5	0.881 (0.598 to 1.065)	0.289
interferon, alpha 8	1.656 (1.165 to 3.123)	0.253
interferon, alpha-inducible protein 27	3.821 (2.876 to 5.078)	0.133
interferon, beta 1, fibroblast	0.876 (0.774 to 1.036)	0.134
interferon, gamma-inducible protein 16	1.328 (0.881 to 2.868)	0.376
interferon, omega 1	0.944 (0.68 to 1.364)	0.754
interferon-induced protein with tetratricopeptide repeats 1	1.178 (0.998 to 1.391)	0.504
interferon-induced protein with tetratricopeptide repeats 4	1.14 (1.071 to 1.213)	0.282
interferon-related developmental regulator 1	0.848 (0.741 to 0.97)	0.436
interleukin 1 receptor, type I	1.04 (0.808 to 1.623)	0.876
interleukin 1 receptor-like 2	0.534 (0.252 to 1.016)	0.147
interleukin 10 receptor, beta	1.03 (0.83 to 1.327)	0.788
interleukin 11 receptor, alpha	1.181 (1.029 to 1.269)	0.138
interleukin 12 receptor, beta 2	1.963 (0.623 to 8.461)	0.245
interleukin 13 receptor, alpha 1	1.048 (1.001 to 1.097)	0.492
interleukin 17 (cytotoxic T-lymphocyte-associated serine esterase 8)	1.407 (1 to 1.98)	0.5
interleukin 18 (interferon-gamma-inducing factor)	1.973 (1.15 to 3.995)	0.207
interleukin 18 receptor 1	1.095 (1.003 to 1.195)	0.488
interleukin 18 receptor accessory protein	1.093 (0.911 to 1.291)	0.47
interleukin 2 receptor, alpha	1.063 (0.941 to 1.132)	0.422
interleukin 3 (colony-stimulating factor, multiple)	2.52 (1.742 to 3.647)	0.242
interleukin 4 receptor	0.78 (0.471 to 1.29)	0.708
interleukin 6 signal transducer (gp130, oncostatin M receptor)	1.237 (1.216 to 1.258)	0.0509
interleukin 9 receptor	0.989 (0.63 to 1.42)	0.952
interleukin enhancer binding factor 2, 45kDa	1.009 (0.521 to 1.371)	0.966
interleukin-1 receptor-associated kinase 1	1.229 (1.002 to 1.736)	0.186
interleukin-1 receptor-associated kinase 2	1.571 (1.267 to 1.949)	0.283
interphotoreceptor matrix proteoglycan 1	1.228 (1.118 to 1.381)	0.0809
intersectin 1 (SH3 domain protein)	0.925 (0.885 to 0.966)	0.323
intracisternal A particle-promoted polypeptide	1.175 (0.7 to 2.083)	0.523
involucrin	0.885 (0.533 to 1.668)	0.665
islet amyloid polypeptide	1.11 (0.821 to 1.527)	0.408
islet cell autoantigen 1, 69kDa	1.099 (0.43 to 3.107)	0.884

isocitrate dehydrogenase 1 (NADP+), soluble	1.094 (0.983 to 1.146)	0.0892
isocitrate dehydrogenase 2 (NADP+), mitochondrial	0.979 (0.651 to 1.292)	0.863
isocitrate dehydrogenase 3 (NAD+) alpha	0.998 (0.523 to 2.243)	0.994
isocitrate dehydrogenase 3 (NAD+) beta	1.235 (0.869 to 3.081)	0.415
isoleucine-tRNA synthetase	1.129 (1.038 to 1.227)	0.385
isopentenyl-diphosphate delta isomerase	0.939 (0.536 to 1.436)	0.815
isovaleryl Coenzyme A dehydrogenase	0.865 (0.416 to 2.041)	0.609
jagged 1 (Alagille syndrome)	0.989 (0.895 to 1.094)	0.931
jerky homolog-like (mouse)	1.191 (0.942 to 1.506)	0.592
jumonji homolog (mouse)	0.911 (0.738 to 1.125)	0.734
jun B proto-oncogene	0.981 (0.471 to 1.416)	0.945
jun D proto-oncogene	0.902 (0.807 to 1.107)	0.42
kallikrein 1, renal/pancreas/salivary	1.056 (0.873 to 1.708)	0.756
kallikrein 10	1.034 (0.969 to 1.104)	0.699
kallikrein 2, prostatic	1.413 (1.189 to 2.152)	0.091
kallikrein 3, (prostate specific antigen)	0.96 (0.752 to 1.225)	0.894
kallikrein 6 (neurosin, zyme)	1.037 (1.029 to 1.046)	0.135
kallikrein 7 (chymotryptic, stratum corneum)	1.031 (0.808 to 1.315)	0.922
kallikrein B, plasma (Fletcher factor) 1	1.931	no replicates
Kallmann syndrome 1 sequence	1.33 (1.049 to 1.686)	0.441
kangai 1 (suppression of tumorigenicity 6, prostate; CD82 antigen (R2 leukocyte antigen, antigen detected by monoclonal and antibody IA4))	0.935 (0.71 to 1.404)	0.778
karyopherin (importin) beta 1	1.024 (0.484 to 1.921)	0.939
karyopherin (importin) beta 2	1.466 (0.79 to 7.63)	0.41
karyopherin (importin) beta 3	0.911 (0.861 to 0.964)	0.348
karyopherin alpha 1 (importin alpha 5)	1.188 (0.515 to 2.738)	0.871
karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	1.282 (0.916 to 1.617)	0.286
karyopherin alpha 3 (importin alpha 4)	1.221 (0.749 to 2.873)	0.55
karyopherin alpha 4 (importin alpha 3)	1.077 (0.646 to 1.903)	0.834
karyopherin alpha 5 (importin alpha 6)	0.912 (0.891 to 0.935)	0.164
kelch domain containing 3	1.103 (1 to 1.246)	0.269
kelch-like 2, Mayven (Drosophila)	1.064 (1 to 1.187)	0.208
Kell blood group	1.131 (0.98 to 1.304)	0.548
keratin 1 (epidermolytic hyperkeratosis)	1.042 (0.947 to 1.114)	0.493
keratin 13	1.116 (1.081 to 1.152)	0.18
keratin 15	1.129 (0.996 to 1.28)	0.511
keratin 16 (focal non-epidermolytic palmoplantar keratoderma)	1.085 (0.916 to 1.199)	0.292
keratin 17	1.037 (0.669 to 1.773)	0.883
keratin 19	0.88 (0.246 to 2.191)	0.799
keratin 2A (epidermal ichthyosis bullosa of Siemens)	0.852 (0.71 to 0.999)	0.107
keratin 6B	1.093 (0.777 to 1.726)	0.555
keratin, cuticle, ultrahigh sulphur 1	1.225 (0.902 to 1.812)	0.264
keratin, hair, acidic, 1	0.905 (0.649 to 1.588)	0.542
keratin, hair, acidic, 2	0.951 (0.619 to 1.459)	0.925
keratin, hair, acidic, 3A	1.36 (0.867 to 2.132)	0.619
keratin, hair, acidic, 5	1.259 (0.829 to 3.803)	0.46
keratin, hair, acidic, 6	1.012	no replicates
keratin, hair, acidic, 7	1.341 (1.039 to 1.731)	0.456
keratin, hair, basic, 3	0.745 (0.459 to 1.621)	0.371
keratin, hair, basic, 5	1.107 (0.682 to 1.458)	0.503
ketohexokinase (fructokinase)	0.941 (0.567 to 1.291)	0.836
KH-type splicing regulatory protein (FUSE binding protein 2)	1.05 (0.953 to 1.158)	0.703
killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 2	0.955 (0.634 to 1.261)	0.789
killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 4	1.396 (0.663 to 2.713)	0.342
killer cell lectin-like receptor subfamily B, member 1	1.155 (0.574 to 3.209)	0.65
killer cell lectin-like receptor subfamily C, member 1	1.107 (0.985 to 1.244)	0.543
killer cell lectin-like receptor subfamily C, member 2	1.08 (0.925 to 1.355)	0.341
killer cell lectin-like receptor subfamily C, member 3	0.959 (0.792 to 1.245)	0.784
killer cell lectin-like receptor subfamily D, member 1	0.856 (0.397 to 1.745)	0.751
kinectin 1 (kinesin receptor)	0.894 (0.73 to 1.171)	0.508
kinesin 2 60/70kDa	1.07 (1 to 1.221)	0.41
kinesin family member 11	0.953 (0.496 to 1.524)	0.899
kinesin family member 22	1.237 (0.672 to 1.683)	0.38
kinesin family member 25	0.898 (0.768 to 1.211)	0.547
kinesin family member 3C	0.829 (0.716 to 1.006)	0.0927
kinesin family member 5A	1.369 (1.292 to 1.451)	0.117
kinesin family member 5B	1.408 (1.348 to 1.471)	0.0805
kinesin family member 5C	0.963 (0.87 to 1.043)	0.389

kinesin family member C3	1.844 (0.982 to 3.461)	0.509
kinesin heavy chain member 2	0.9 (0.785 to 1.118)	0.304
kininogen	1.057 (1.035 to 1.078)	0.227
Kruppel-like factor 12	0.403 (0.0156 to 4.493)	0.645
Kruppel-like factor 5 (intestinal)	1.42 (0.749 to 4.302)	0.43
kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	1.968 (1.126 to 4.513)	0.251
L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain	0.829 (0.519 to 1.722)	0.517
lactalbumin, alpha-	1.216 (0.679 to 2.694)	0.574
lactase	0.968 (0.874 to 1.072)	0.804
lactate dehydrogenase A	0.884 (0.55 to 1.291)	0.495
lactate dehydrogenase B	1.876 (1.234 to 2.852)	0.374
lactate dehydrogenase C	0.803 (0.364 to 1.424)	0.646
lactotransferrin	1.243 (1.175 to 1.314)	0.16
ladinin 1	0.787 (0.37 to 1.22)	0.431
lamin A/C	1.465 (1.16 to 1.851)	0.349
lamin B receptor	0.873 (0.439 to 1.3)	0.732
lamin B1	1.159 (0.953 to 1.518)	0.249
laminin, alpha 2 (merosin, congenital muscular dystrophy)	0.943 (0.734 to 1.226)	0.626
laminin, alpha 3	2.51 (2.172 to 2.9)	0.0993
laminin, alpha 4	0.475 (0.116 to 1.026)	0.402
laminin, beta 3	1.193 (0.673 to 2.425)	0.56
laminin, gamma 2	1.101 (0.845 to 1.518)	0.408
lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	1.109 (0.963 to 1.277)	0.597
latent transforming growth factor beta binding protein 1	0.928 (0.733 to 1.445)	0.671
latent transforming growth factor beta binding protein 2	1.051 (0.724 to 1.794)	0.766
lectin, galactoside-binding, soluble, 1 (galectin 1)	1.213 (0.727 to 1.896)	0.4
lectin, galactoside-binding, soluble, 3 (galectin 3)	1.154 (0.694 to 1.92)	0.825
lectin, galactoside-binding, soluble, 3 binding protein	1.311 (1.089 to 1.579)	0.383
lectin, galactoside-binding, soluble, 4 (galectin 4)	1.093 (0.695 to 1.914)	0.745
lectin, galactoside-binding, soluble, 7 (galectin 7)	0.957 (0.894 to 1.001)	0.199
lectin, galactoside-binding, soluble, 8 (galectin 8)	0.848 (0.412 to 1.459)	0.582
lectin, galactoside-binding, soluble, 9 (galectin 9)	1.033 (0.79 to 1.427)	0.809
lectin, mannose-binding, 1	0.788 (0.341 to 1.281)	0.468
leptin (obesity homolog, mouse)	0.878 (0.511 to 1.51)	0.851
lethal giant larvae homolog 1 (Drosophila)	1.269 (1.037 to 1.552)	0.447
lethal giant larvae homolog 2 (Drosophila)	0.634 (0.225 to 1.072)	0.285
leucine rich repeat neuronal 4	1.207 (0.978 to 1.728)	0.406
leucyl/cystinyl aminopeptidase	0.957 (0.731 to 1.255)	0.898
leukemia inhibitory factor receptor	0.781 (0.495 to 1.068)	0.399
leukocyte cell-derived chemotaxin 2	0.917 (0.615 to 1.367)	0.676
leukocyte-associated Ig-like receptor 1	0.7 (0.588 to 0.877)	0.0946
leukocyte-associated Ig-like receptor 2	1.348 (1.223 to 1.486)	0.201
leukotriene A4 hydrolase	0.827 (0.659 to 1.039)	0.559
leukotriene B4 receptor	1.245 (0.889 to 1.92)	0.436
leukotriene C4 synthase	0.768 (0.199 to 1.717)	0.611
LIM and senescent cell antigen-like domains 1	1.807 (1.452 to 2.25)	0.226
LIM and SH3 protein 1	1.449 (1.172 to 1.793)	0.331
LIM domain containing preferred translocation partner in lipoma	1.189 (0.944 to 1.781)	0.483
LIM domain kinase 1	0.994 (0.936 to 1.054)	0.932
LIM domain kinase 2	1.576 (1.042 to 2.385)	0.47
LIM domain only 1 (rhombotin 1)	0.741 (0.585 to 0.939)	0.425
LIM domain only 2 (rhombotin-like 1)	0.969 (0.825 to 1.234)	0.82
LIM domain only 6	0.772 (0.663 to 0.899)	0.339
LIM domain only 7	1.61 (1.415 to 1.831)	0.168
LIM domain protein	0.861 (0.712 to 1.041)	0.576
LIM homeobox protein 1	1.134 (0.826 to 1.494)	0.254
LIM homeobox transcription factor 1, beta	1.246 (1.06 to 1.39)	0.0367
limbic system-associated membrane protein	1.09 (0.905 to 1.313)	0.724
lipase A, lysosomal acid, cholesterol esterase (Wolman disease)	1.244 (0.775 to 1.54)	0.147
lipase, gastric	0.859 (0.711 to 1.038)	0.57
lipase, hepatic	1.094 (0.717 to 1.498)	0.517
lipase, hormone-sensitive	1.465 (0.858 to 2.205)	0.305
lipocalin 1 (protein migrating faster than albumin, tear prealbumin)	1.313 (0.9 to 2.17)	0.246
lipocalin 2 (oncogene 24p3)	0.732 (0.384 to 1.273)	0.295
lipoprotein lipase	1.052 (0.897 to 1.195)	0.46
lipoprotein, Lp(a)	1.653 (1.211 to 1.908)	0.0174
loricrin	1.103 (0.853 to 1.481)	0.327
low density lipoprotein receptor-related protein 3	1.149 (1.122 to 1.176)	0.108
low density lipoprotein receptor-related protein 6	2.123 (1.75 to 2.576)	0.16
low density lipoprotein receptor-related protein 8, apolipoprotein e	0.909	no replicates

receptor		
lumican	0.822 (0.678 to 1.008)	0.229
luteinizing hormone/choriogonadotropin receptor	1.159 (0.878 to 1.529)	0.689
Lutheran blood group (Auberger b antigen included)	1.596 (1.336 to 1.906)	0.232
lymphocyte antigen 6 complex, locus H	1.007 (0.904 to 1.123)	0.957
lymphocyte antigen 75	0.975 (0.679 to 1.513)	0.889
lymphocyte cytosolic protein 1 (L-plastin)	0.954 (0.706 to 2.236)	0.879
lymphocyte-activation gene 3	1.352 (0.94 to 1.943)	0.559
lymphocyte-specific protein tyrosine kinase	1.613 (1.328 to 2.146)	0.0816
lymphoid nuclear protein related to AF4	0.911 (0.497 to 1.246)	0.687
lymphoid-restricted membrane protein	0.365 (0.0583 to 1.013)	0.386
lysosomal-associated membrane protein 1	0.906 (0.476 to 1.736)	0.816
lysosomal-associated membrane protein 2	0.413 (0.0818 to 1.188)	0.395
lysozyme (renal amyloidosis)	1.047 (0.748 to 1.457)	0.744
lysyl oxidase	1.411 (0.802 to 3.237)	0.502
lysyl oxidase-like 1	1.171 (1.054 to 1.338)	0.154
lysyl oxidase-like 2	1.414 (1.111 to 2.069)	0.103
lysyl-tRNA synthetase	0.928 (0.736 to 1.171)	0.803
mab-21-like 1 (C. elegans)	1.157 (0.984 to 1.36)	0.533
macrophage scavenger receptor 1	0.864 (0.575 to 1.432)	0.508
MAD, mothers against decapentaplegic homolog 1 (Drosophila)	0.82 (0.76 to 0.884)	0.231
MAD, mothers against decapentaplegic homolog 2 (Drosophila)	0.688 (0.196 to 1.336)	0.318
MAD, mothers against decapentaplegic homolog 3 (Drosophila)	1.308 (0.869 to 1.997)	0.262
MAD, mothers against decapentaplegic homolog 5 (Drosophila)	1.183 (1.126 to 1.242)	0.18
MAD, mothers against decapentaplegic homolog 6 (Drosophila)	1.293 (0.865 to 2.762)	0.279
MAD, mothers against decapentaplegic homolog 7 (Drosophila)	1.077 (0.893 to 1.298)	0.76
MAD, mothers against decapentaplegic homolog 9 (Drosophila)	0.728 (0.408 to 1.016)	0.222
MAD1 mitotic arrest deficient-like 1 (yeast)	1.087 (1.013 to 1.167)	0.449
MAD2 mitotic arrest deficient-like 1 (yeast)	1.027 (0.786 to 1.336)	0.841
MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)	1.229 (0.916 to 1.646)	0.184
MADS box transcription enhancer factor 2, polypeptide B (myocyte enhancer factor 2B)	0.976 (0.964 to 0.989)	0.319
MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)	1.241 (0.922 to 1.891)	0.141
MADS box transcription enhancer factor 2, polypeptide D (myocyte enhancer factor 2D)	1.137 (0.926 to 1.59)	0.527
mago-nashi homolog, proliferation-associated (Drosophila)	0.793	no replicates
major histocompatibility complex, class I, C	0.943 (0.806 to 1.119)	0.453
major histocompatibility complex, class I, E	1.038 (0.855 to 1.248)	0.765
major histocompatibility complex, class II, DM alpha	1.226 (1.197 to 1.256)	0.0745
major histocompatibility complex, class II, DM beta	1.245 (0.648 to 2.104)	0.492
major histocompatibility complex, class II, DO alpha	0.964 (0.86 to 1.139)	0.623
major histocompatibility complex, class II, DO beta	1.079 (0.793 to 1.285)	0.53
major histocompatibility complex, class II, DP beta 1	1.871 (1.321 to 3.36)	0.167
major histocompatibility complex, class II, DQ alpha 1	1.191 (0.779 to 2.26)	0.425
major histocompatibility complex, class II, DQ beta 1	0.871 (0.535 to 1.72)	0.646
major histocompatibility complex, class II, DR beta 1	0.929 (0.924 to 0.934)	0.0439
major histocompatibility complex, class I-related	1.611 (0.577 to 11.77)	0.531
malate dehydrogenase 1, NAD (soluble)	0.667 (0.614 to 0.725)	0.129
malate dehydrogenase 2, NAD (mitochondrial)	1.022 (0.869 to 1.114)	0.735
malic enzyme 1, NADP(+)-dependent, cytosolic	1.099 (0.811 to 1.397)	0.463
malic enzyme 2, NAD(+)-dependent, mitochondrial	0.947 (0.769 to 1.059)	0.653
mannan-binding lectin serine protease 1 (C4/C2 activating component of Ra-reactive factor)	1.113 (0.781 to 1.616)	0.564
mannose phosphate isomerase	1.384 (1.232 to 1.632)	0.0615
mannose receptor, C type 1	1.024 (0.907 to 1.156)	0.878
mannose-6-phosphate receptor (cation dependent)	1.578 (1.438 to 1.732)	0.128
mannose-binding lectin (protein C) 2, soluble (opsonic defect)	0.924 (0.487 to 1.612)	0.74
mannosidase, alpha, class 1A, member 1	0.98 (0.759 to 1.258)	0.871
mannosidase, alpha, class 2A, member 2	0.938 (0.702 to 1.436)	0.621
mannosidase, alpha, class 2C, member 1	0.844 (0.663 to 1.05)	0.2
mannosidase, beta A, lysosomal	0.92 (0.785 to 1.109)	0.495
mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase	1.154 (0.735 to 1.514)	0.3
mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase	1.134 (0.96 to 1.376)	0.354
mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase	1.175 (0.903 to 2.301)	0.399
mannosyl (beta-1,4-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase	0.789 (0.564 to 1.266)	0.433

MAP kinase-interacting serine/threonine kinase 1	1.137 (0.947 to 1.366)	0.61
MAP/microtubule affinity-regulating kinase 2	0.717 (0.644 to 0.759)	0.00307
MAP-kinase activating death domain	1.405 (1.286 to 1.534)	0.162
matrilin 1, cartilage matrix protein	0.843 (0.694 to 1.122)	0.244
matrilin 4	0.978 (0.91 to 1.051)	0.81
matrix Gla protein	0.659 (0.655 to 0.662)	0.00909
matrix metalloproteinase 14 (membrane-inserted)	0.98 (0.558 to 1.538)	0.909
matrix metalloproteinase 23A	1.32 (1.065 to 1.786)	0.216
matrix metalloproteinase-like 1	1.287 (0.786 to 1.952)	0.316
MAX dimerization protein 1	1.351 (0.98 to 1.595)	0.0703
MAX interacting protein 1	0.904 (0.648 to 1.369)	0.448
MAX protein	1.226 (0.864 to 2.9)	0.53
MCF.2 cell line derived transforming sequence	1.137 (0.816 to 1.741)	0.534
MCM2 minichromosome maintenance deficient 2, mitotin (S. cerevisiae)	0.534 (0.347 to 0.821)	0.383
MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae)	0.971 (0.9 to 1.049)	0.77
Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	0.876 (0.723 to 1.136)	0.429
Mediterranean fever	0.89 (0.659 to 1.181)	0.356
megakaryocyte-associated tyrosine kinase	0.926 (0.662 to 1.295)	0.856
Meis1, myeloid ecotropic viral integration site 1 homolog (mouse)	1.195 (0.457 to 2.849)	0.733
melan-A	1.012 (0.869 to 1.178)	0.951
melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)	1.301 (0.802 to 2.113)	0.683
melanocortin 2 receptor (adrenocorticotropic hormone)	1.037 (0.729 to 1.571)	0.801
melanocortin 4 receptor	0.878 (0.869 to 0.887)	0.0483
melanocortin 5 receptor	2.604 (0.971 to 6.985)	0.51
melanoma antigen, family A, 1 (directs expression of antigen MZ2-E)	0.957 (0.777 to 1.136)	0.624
melanoma antigen, family A, 11	1.156 (0.83 to 1.617)	0.43
melanoma antigen, family A, 2	0.834 (0.552 to 1.223)	0.512
melanoma antigen, family A, 4	0.953 (0.806 to 1.143)	0.638
melanoma antigen, family A, 8	1.085 (1.014 to 1.133)	0.0412
melanoma antigen, family A, 9	1.184 (0.99 to 1.416)	0.13
melanoma antigen, family B, 1	0.984 (0.963 to 1.005)	0.582
melanoma antigen, family B, 2	1.251 (0.98 to 1.598)	0.528
melanoma antigen, family B, 4	1.933 (0.868 to 4.305)	0.562
melatonin receptor 1B	1.263 (0.783 to 2.882)	0.364
membrane component, chromosome 11, surface marker 1	1.394 (1.285 to 1.512)	0.153
membrane component, chromosome 17, surface marker 2 (ovarian carcinoma antigen CA125)	0.985 (0.73 to 1.696)	0.941
membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)	0.947 (0.684 to 1.31)	0.894
membrane protein, palmitoylated 1, 55kDa	0.966 (0.655 to 1.263)	0.83
membrane protein, palmitoylated 3 (MAGUK p55 subfamily member 3)	1.259 (1.254 to 1.264)	0.0109
membrane-bound transcription factor protease, site 1	1.364 (0.955 to 1.948)	0.544
membrane-spanning 4-domains, subfamily A, member 2 (Fc fragment of IgE, high affinity I, receptor for; beta polypeptide)	0.797 (0.509 to 1.236)	0.379
membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)	1.131 (1.072 to 1.194)	0.262
menage a trois 1 (CAK assembly factor)	0.881 (0.553 to 1.162)	0.644
meningioma (disrupted in balanced translocation) 1	1.151 (0.848 to 1.413)	0.315
meningioma expressed antigen 6 (coiled-coil proline-rich)	0.786 (0.644 to 0.96)	0.441
meprin A, alpha (PABA peptide hydrolase)	0.895 (0.568 to 1.226)	0.592
meprin A, beta	1.362 (0.764 to 2.157)	0.196
mercaptopyruvate sulfurtransferase	0.809 (0.712 to 0.92)	0.346
mesenchyme homeo box 1	0.737 (0.307 to 1.23)	0.392
mesenchyme homeo box 2 (growth arrest-specific homeo box)	0.891 (0.66 to 1.117)	0.267
mesoderm specific transcript homolog (mouse)	0.912 (0.605 to 1.339)	0.637
metallothionein 2A	1.981 (1.279 to 4.245)	0.216
metaxin 1	1.481 (1.288 to 1.703)	0.218
methionine adenosyltransferase I, alpha	0.958 (0.896 to 1.044)	0.265
methionine adenosyltransferase II, alpha	1.781 (1.088 to 4.628)	0.35
methionine-tRNA synthetase	1.048 (0.8 to 1.376)	0.716
methyl CpG binding protein 2 (Rett syndrome)	0.532 (0.328 to 0.711)	0.0326
methylmalonyl Coenzyme A mutase	0.899 (0.335 to 1.677)	0.782
methylthioadenosine phosphorylase	0.82 (0.551 to 1.212)	0.343
mevalonate (diphospho) decarboxylase	1.025 (0.84 to 1.136)	0.752
mevalonate kinase (mevalonic aciduria)	2.02 (1.66 to 2.458)	0.173
MHC class I polypeptide-related sequence A	0.944 (0.74 to 1.076)	0.42
MHC class I polypeptide-related sequence B	0.954 (0.759 to 1.082)	0.497
MHC class II HLA DR5 DR-beta-chain	0.375 (0.0493 to 0.993)	0.248

MHC class II transactivator	0.959 (0.734 to 1.26)	0.812
Microfibril-associated glycoprotein-2	1.063 (0.64 to 1.889)	0.863
microfibrillar-associated protein 1	1.034 (0.647 to 1.713)	0.879
microfibrillar-associated protein 2	1.07 (1.056 to 1.085)	0.127
microfibrillar-associated protein 3	0.914 (0.408 to 2.047)	0.865
microseminoprotein, beta-	1.592 (1.128 to 2.155)	0.0113
microsomal glutathione S-transferase 3	1.018 (0.661 to 1.307)	0.885
microsomal NAD+-dependent retinol dehydrogenase 4	1.163 (0.901 to 1.47)	0.337
microsomal triglyceride transfer protein (large polypeptide, 88kDa)	0.986 (0.952 to 1.022)	0.762
microtubule-associated protein 1A	0.652 (0.549 to 0.729)	0.0395
microtubule-associated protein 1B	1.071 (0.939 to 1.394)	0.504
microtubule-associated protein 2	0.807 (0.803 to 0.811)	0.0142
microtubule-associated protein 4	1.337 (1.184 to 1.511)	0.253
microtubule-associated protein tau	0.909 (0.604 to 1.307)	0.549
midkine (neurite growth-promoting factor 2)	1.267 (1.214 to 1.322)	0.114
midline 1 (Opitz/BBB syndrome)	1.503 (1.43 to 1.58)	0.0775
milk fat globule-EGF factor 8 protein	1.447 (1.14 to 2.26)	0.24
mitochondrial intermediate peptidase	0.649 (0.454 to 0.894)	0.0874
mitochondrial ribosomal protein L12	1.724 (1.593 to 1.866)	0.0917
mitochondrial ribosomal protein L40	0.97 (0.42 to 3.183)	0.965
mitochondrial translational initiation factor 2	1.446 (1.132 to 1.847)	0.373
mitogen-activated protein kinase 10	1.003 (0.937 to 1.073)	0.973
mitogen-activated protein kinase 11	0.761 (0.421 to 1.195)	0.47
mitogen-activated protein kinase 12	1.384 (0.885 to 1.972)	0.302
mitogen-activated protein kinase 13	1.377 (0.737 to 2.573)	0.699
mitogen-activated protein kinase kinase kinase 10	1.032 (0.614 to 1.478)	0.882
mitogen-activated protein kinase kinase kinase 4	1.344 (0.955 to 1.891)	0.546
mitogen-activated protein kinase kinase kinase 7	1.287 (0.91 to 1.819)	0.6
mitogen-activated protein kinase kinase kinase kinase 2	1.235 (1.07 to 1.425)	0.38
mitogen-activated protein kinase kinase kinase kinase 3	1.654	no replicates
mitogen-activated protein kinase-activated protein kinase 3	1.054 (0.57 to 2.084)	0.877
mitogen-activated protein kinase-activated protein kinase 5	1.116 (0.864 to 1.406)	0.518
moesin	1.069 (0.77 to 2.019)	0.854
molybdenum cofactor synthesis 2	1.246 (0.84 to 1.664)	0.394
motilin	1.68 (0.978 to 2.87)	0.0998
MRE11 meiotic recombination 11 homolog A (S. cerevisiae)	1.147 (0.948 to 1.387)	0.603
msh homeo box homolog 2 (Drosophila)	0.682 (0.452 to 1.027)	0.521
mucin 1, transmembrane	1.172 (0.939 to 1.419)	0.318
mucin 2, intestinal/tracheal	1.129 (0.763 to 2.344)	0.595
multiple endocrine neoplasia I	0.907 (0.69 to 1.362)	0.431
multiple PDZ domain protein	1.334 (1.236 to 1.44)	0.164
musashi homolog 1 (Drosophila)	1.349 (1.175 to 1.549)	0.276
muscle, skeletal, receptor tyrosine kinase	1.322 (1.009 to 1.73)	0.489
mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)	1.082 (0.853 to 1.372)	0.796
mutS homolog 3 (E. coli)	0.957 (0.79 to 1.16)	0.858
mutS homolog 4 (E. coli)	1.241 (1.091 to 1.412)	0.343
mutS homolog 5 (E. coli)	1.066 (0.736 to 1.437)	0.589
mutS homolog 6 (E. coli)	1.307 (1.265 to 1.35)	0.0765
myelin basic protein	1.197 (1.064 to 1.347)	0.369
myelin protein zero (Charcot-Marie-Tooth neuropathy 1B)	1.038 (0.797 to 1.352)	0.911
myelin-associated oligodendrocyte basic protein	0.832 (0.784 to 0.92)	0.068
myeloid differentiation primary response gene (88)	0.932 (0.691 to 1.182)	0.698
myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	2.312 (1.103 to 4.848)	0.461
myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 1	1.104 (1.097 to 1.112)	0.0415
myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10	1.2	no replicates
myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 2	1.099 (0.963 to 1.478)	0.286
myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 3	1.082 (0.927 to 1.196)	0.152
myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 4	1.447 (0.974 to 2.148)	0.522
myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 6	1	no replicates
myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 7	0.849 (0.714 to 0.953)	0.203
myeloid/lymphoid or mixed-lineage leukemia 2	0.858 (0.542 to 1.326)	0.613
myeloperoxidase	1.139 (0.95 to 1.52)	0.202
myeloproliferative leukemia virus oncogene	0.992 (0.68 to 1.321)	0.962

myocilin, trabecular meshwork inducible glucocorticoid response	1.241 (0.925 to 1.92)	0.259
MyoD family inhibitor	1.196 (0.795 to 1.808)	0.368
myogenic factor 3	1.053 (0.52 to 2.38)	0.852
myogenic factor 5	0.994 (0.656 to 1.365)	0.971
myogenin (myogenic factor 4)	0.705 (0.557 to 1.116)	0.114
myomesin 1 (skelemin) 185kDa	0.836 (0.694 to 1.007)	0.513
myosin binding protein C, cardiac	1.475 (0.99 to 2.668)	0.169
myosin binding protein C, fast type	0.938 (0.738 to 1.046)	0.491
myosin binding protein C, slow type	1.55 (1.414 to 1.699)	0.131
myosin binding protein H	1.048 (0.807 to 1.321)	0.687
myosin IA	1.149 (0.946 to 1.422)	0.36
myosin IE	0.998 (0.575 to 1.526)	0.991
myosin IXA	1.171 (0.998 to 1.422)	0.267
myosin IXB	1.104 (1.073 to 1.136)	0.177
myosin light chain 1 slow a	0.709 (0.619 to 0.813)	0.24
myosin VI	1.037 (0.389 to 4.323)	0.964
myosin VIIA (Usher syndrome 1B (autosomal recessive, severe))	1.502 (1.131 to 1.995)	0.388
myosin, heavy polypeptide 1, skeletal muscle, adult	1.045 (0.704 to 1.594)	0.816
myosin, heavy polypeptide 7, cardiac muscle, beta	0.843 (0.783 to 0.906)	0.257
myosin, heavy polypeptide 8, skeletal muscle, perinatal	1.004 (0.888 to 1.135)	0.98
myosin, light polypeptide 2, regulatory, cardiac, slow	1.016 (0.834 to 1.285)	0.912
myosin, light polypeptide 3, alkali; ventricular, skeletal, slow	2.154 (0.874 to 5.334)	0.279
myosin, light polypeptide 4, alkali; atrial, embryonic	1.229 (1.175 to 1.286)	0.136
myosin, light polypeptide 5, regulatory	1.165 (0.996 to 1.318)	0.0477
myosin, light polypeptide kinase	0.924 (0.514 to 1.326)	0.736
myristoylated alanine-rich protein kinase C substrate	1.092 (0.761 to 1.409)	0.684
myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	1.33 (1.249 to 1.415)	0.137
N-acetylgalactosaminidase, alpha-	0.783 (0.672 to 0.904)	0.0278
N-acetylglucosaminidase, alpha- (Sanfilippo disease IIIB)	1.232 (0.918 to 1.85)	0.157
N-acetyltransferase 2 (arylamine N-acetyltransferase)	1.042 (1.029 to 1.055)	0.187
N-acylaminoacyl-peptide hydrolase	0.929 (0.807 to 1.069)	0.691
N-acylsphingosine amidohydrolase (acid ceramidase) 1	1.59 (1.023 to 3.81)	0.118
NAD(P)H dehydrogenase, quinone 2	0.894 (0.749 to 0.979)	0.331
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1, 7.5kDa	0.942 (0.723 to 1.228)	0.86
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10, 42kDa	0.53 (0.237 to 0.821)	0.256
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8kDa	0.905 (0.899 to 0.912)	0.0486
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 3, 9kDa	0.965 (0.865 to 1.042)	0.493
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4, 9kDa	1.094 (0.855 to 1.393)	0.591
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa	1.17 (0.779 to 1.651)	0.303
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 7, 14.5kDa	1.1 (0.717 to 2.16)	0.806
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 9, 39kDa	1.093 (0.728 to 1.48)	0.714
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 1, 7kDa	0.615 (0.503 to 0.751)	0.249
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10, 22kDa	1.081 (0.527 to 1.834)	0.785
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2, 8kDa	1.501 (0.953 to 2.181)	0.236
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa	0.988 (0.793 to 1.18)	0.898
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa	0.824 (0.268 to 1.392)	0.647
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5, 16kDa	0.797 (0.287 to 1.156)	0.429
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa	1.295 (1.246 to 1.345)	0.0938
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, 19kDa	1.002 (0.612 to 1.642)	0.997
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9, 22kDa	1.052 (0.705 to 1.57)	0.92
NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1, 6kDa	0.883 (0.804 to 1.019)	0.229
NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 2, 14.5kDa	0.872 (0.762 to 0.998)	0.495
NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase)	0.723 (0.393 to 1.363)	0.211
NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase)	0.996 (0.958 to 1.035)	0.929
NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30kDa (NADH-coenzyme Q reductase)	1.171 (1.148 to 1.194)	0.0796
NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)	1.082 (0.802 to 1.726)	0.772
NADH dehydrogenase (ubiquinone) Fe-S protein 5, 15kDa (NADH-coenzyme Q reductase)	1.138 (0.926 to 1.398)	0.644
NADH dehydrogenase (ubiquinone) Fe-S protein 6, 13kDa (NADH-coenzyme Q reductase)	0.858 (0.718 to 1.024)	0.546
NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase)	0.78 (0.668 to 0.872)	0.0242
NADH dehydrogenase (ubiquinone) flavoprotein 1, 51kDa	1.499 (1.058 to 2.125)	0.452
nardilysin (N-arginine dibasic convertase)	1.266 (1.182 to 1.423)	0.057
nascent-polypeptide-associated complex alpha polypeptide	0.835 (0.578 to 1.442)	0.428
natriuretic peptide precursor B	0.871 (0.476 to 1.591)	0.856

natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	0.693 (0.576 to 0.863)	0.00936
natriuretic peptide receptor B/guanylate cyclase B (atrionatriuretic peptide receptor B)	0.581 (0.335 to 1.007)	0.504
natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)	0.898 (0.857 to 0.94)	0.257
natural killer cell group 7 sequence	1.085 (0.69 to 1.353)	0.632
natural killer-tumor recognition sequence	1.641 (1.272 to 2.457)	0.136
NCK adaptor protein 2	1.019 (0.778 to 1.33)	0.884
N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 2	0.908 (0.674 to 1.223)	0.8
necdin homolog (mouse)	1.361 (0.751 to 6.079)	0.464
NEL-like 1 (chicken)	0.7 (0.411 to 0.969)	0.314
NEL-like 2 (chicken)	1.485 (1.132 to 1.947)	0.383
neogenin homolog 1 (chicken)	1.146 (1.041 to 1.262)	0.392
nephroblastoma overexpressed gene	1.185 (0.963 to 1.357)	0.248
nephronophthisis 1 (juvenile)	0.991 (0.772 to 1.273)	0.939
nephrosis 1, congenital, Finnish type (nephrin)	1.142 (0.731 to 1.466)	0.613
nerve growth factor receptor (TNFR superfamily, member 16)	0.8 (0.296 to 1.259)	0.439
nescient helix loop helix 2	1.145 (1.046 to 1.255)	0.376
N-ethylmaleimide-sensitive factor attachment protein, alpha	1.092 (0.489 to 1.415)	0.686
N-ethylmaleimide-sensitive factor attachment protein, gamma	0.8 (0.428 to 1.225)	0.557
neural cell adhesion molecule 2	0.84 (0.546 to 1.069)	0.503
neural precursor cell expressed, developmentally down-regulated 8	0.66 (0.45 to 0.968)	0.474
neuroblastoma, suppression of tumorigenicity 1	1.071 (0.73 to 1.866)	0.682
neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)	1.046 (0.827 to 1.241)	0.683
neurofilament 3 (150kDa medium)	1.179 (1.046 to 1.329)	0.399
neurofilament, light polypeptide 68kDa	1.378 (1.104 to 1.719)	0.385
neurogenic differentiation 1	3.575	no replicates
neurogenic differentiation 2	1.72 (1.054 to 3.534)	0.278
neurogenin 1	0.682 (0.511 to 0.964)	0.0659
neuronal cell adhesion molecule	0.989 (0.672 to 1.419)	0.949
neuronal PAS domain protein 1	1.205 (0.885 to 1.904)	0.333
neuronal PAS domain protein 2	1.288 (1.192 to 1.445)	0.0497
neuronal pentraxin I	0.719 (0.282 to 1.235)	0.555
neuro-oncological ventral antigen 1	1.493	no replicates
neuro-oncological ventral antigen 2	0.674 (0.605 to 0.825)	0.0598
neuropeptide FF-amide peptide precursor	1.281 (0.944 to 1.901)	0.354
neuropeptide Y	1.332 (0.852 to 2.002)	0.249
neuropeptide Y receptor Y2	1.228 (1.035 to 1.456)	0.442
neuropeptide Y receptor Y5	0.749	no replicates
neuropeptide Y receptor Y6 (pseudogene)	1.089 (0.951 to 1.226)	0.369
neurotensin receptor 1 (high affinity)	0.977 (0.904 to 1.128)	0.777
neurotrophin 6, alpha pseudogene	1.109 (0.96 to 1.256)	0.157
neurturin	1.507 (1.204 to 2.101)	0.137
neutral sphingomyelinase (N-SMase) activation associated factor	1.514 (0.967 to 2.239)	0.125
neutrophil cytosolic factor 1 (47kDa, chronic granulomatous disease, autosomal 1)	1.354 (0.903 to 2.029)	0.591
neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2)	0.959 (0.637 to 1.282)	0.733
neutrophil cytosolic factor 4, 40kDa	1.393 (1.218 to 1.593)	0.245
NGFI-A binding protein 1 (EGR1 binding protein 1)	0.921 (0.402 to 1.449)	0.732
NHP2 non-histone chromosome protein 2-like 1 (S. cerevisiae)	1.009 (0.593 to 1.958)	0.982
nicotinamide N-methyltransferase	1.023 (0.615 to 2.302)	0.922
Niemann-Pick disease, type C1	1.541 (1.053 to 2.255)	0.46
NIMA (never in mitosis gene a)-related kinase 2	1.462 (0.799 to 2.392)	0.262
nipsnap homolog 1 (C. elegans)	1.335 (0.982 to 1.519)	0.0674
nitrilase 1	1.004 (0.836 to 1.204)	0.971
NK2 transcription factor related, locus 2 (Drosophila)	0.979 (0.922 to 1.038)	0.777
NK2 transcription factor related, locus 5 (Drosophila)	0.717 (0.376 to 0.98)	0.226
NK3 transcription factor related, locus 1 (Drosophila)	0.919 (0.884 to 0.955)	0.272
NK6 transcription factor related, locus 1 (Drosophila)	0.638 (0.267 to 1.665)	0.375
N-methylpurine-DNA glycosylase	1.722	no replicates
non-metastatic cells 2, protein (NM23B) expressed in	1.189 (1.177 to 1.201)	0.0372
non-metastatic cells 5, protein expressed in (nucleoside-diphosphate kinase)	1.17 (1.104 to 1.24)	0.225
Norrie disease (pseudoglioma)	0.837 (0.176 to 3.969)	0.927
Notch homolog 3 (Drosophila)	1.271 (1.07 to 1.399)	0.0285
N-sulfoglucosamine sulfohydrolase (sulfamidase)	1.203 (0.942 to 1.537)	0.587
nth endonuclease III-like 1 (E. coli)	1.163 (0.876 to 1.646)	0.331
nuclear antigen Sp100	1.621 (1.134 to 2.318)	0.405

nuclear autoantigenic sperm protein (histone-binding)	0.979 (0.85 to 1.127)	0.903
nuclear factor (erythroid-derived 2), 45kDa	0.804 (0.766 to 0.843)	0.138
nuclear factor (erythroid-derived 2)-like 2	0.745 (0.368 to 1.416)	0.529
nuclear factor I/B	0.824 (0.68 to 0.999)	0.498
nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta	0.905 (0.347 to 1.521)	0.854
nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon	1.184 (0.898 to 1.561)	0.651
nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1	1.321 (1.144 to 1.827)	0.0867
nuclear factor, interleukin 3 regulated	1.006 (0.98 to 1.032)	0.865
nuclear protein, ataxia-telangiectasia locus	0.546 (0.017 to 2.854)	0.641
nuclear protein, marker for differentiated aortic smooth muscle and down-regulated with vascular injury	1.24	no replicates
nuclear receptor coactivator 1	1.032 (0.881 to 1.139)	0.734
nuclear receptor coactivator 3	0.629 (0.266 to 1.416)	0.159
nuclear receptor interacting protein 1	0.826 (0.818 to 0.835)	0.0341
nuclear receptor subfamily 0, group B, member 1	2.328 (2.138 to 2.536)	0.0641
nuclear receptor subfamily 2, group C, member 1	0.812 (0.179 to 2.186)	0.645
nuclear receptor subfamily 2, group C, member 2	0.94 (0.87 to 1.015)	0.568
nuclear receptor subfamily 2, group E, member 1	1.286 (1.166 to 1.419)	0.237
nuclear receptor subfamily 2, group F, member 1	0.79 (0.578 to 1.08)	0.589
nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	1.023 (0.952 to 1.069)	0.59
nuclear receptor subfamily 3, group C, member 2	0.569 (0.174 to 1.325)	0.18
nuclear receptor subfamily 4, group A, member 1	1.404 (0.996 to 2.552)	0.225
nuclear receptor subfamily 4, group A, member 2	1.261 (1.223 to 1.301)	0.0844
nuclear receptor subfamily 5, group A, member 1	1.148 (0.849 to 2.4)	0.615
nuclear receptor subfamily 5, group A, member 2	0.881 (0.774 to 0.999)	0.118
nuclear receptor subfamily 6, group A, member 1	0.959 (0.957 to 0.962)	0.0461
nuclear transcription factor Y, alpha	0.714 (0.606 to 0.84)	0.0196
nuclear transcription factor Y, beta	1.321 (1.053 to 1.656)	0.435
nuclear VCP-like	1.02 (1.008 to 1.032)	0.347
nuclease sensitive element binding protein 1	1.734 (1.095 to 2.747)	0.443
nucleobindin 2	1.251 (0.783 to 1.998)	0.716
nucleolar protein 4	1.33 (0.801 to 1.881)	0.386
nucleolin	1.085 (0.862 to 1.477)	0.662
nucleoporin 88kDa	1.351 (1.293 to 1.411)	0.0914
nucleoporin 98kDa	0.896 (0.846 to 0.949)	0.309
nucleoside phosphorylase	0.651 (0.227 to 0.946)	0.184
nucleosome assembly protein 1-like 1	1.192 (0.671 to 1.672)	0.453
nucleosome assembly protein 1-like 3	0.885 (0.496 to 1.2)	0.59
nucleosome assembly protein 1-like 4	0.759 (0.59 to 1.103)	0.149
nucleotide binding protein 1 (MinD homolog, E. coli)	1.358 (1.281 to 1.439)	0.12
nudix (nucleoside diphosphate linked moiety X)-type motif 1	0.889 (0.869 to 0.91)	0.124
nudix (nucleoside diphosphate linked moiety X)-type motif 2	2.209 (2.11 to 2.313)	0.037
numb homolog (Drosophila)	1.068 (0.551 to 1.822)	0.868
occludin	0.948 (0.601 to 1.256)	0.782
ocular albinism 1 (Nettleship-Falls)	0.729 (0.542 to 0.964)	0.197
oculocerebrorenal syndrome of Lowe	1.12 (1.028 to 1.221)	0.412
oculocutaneous albinism II (pink-eye dilution homolog, mouse)	1.028 (0.976 to 1.113)	0.565
olfactomedin 1	1.049 (1.042 to 1.055)	0.0828
olfactory marker protein	0.537 (0.283 to 1.017)	0.508
olfactory receptor, family 1, subfamily D, member 2	0.897 (0.779 to 1.122)	0.439
olfactory receptor, family 3, subfamily A, member 1	0.984 (0.912 to 1.053)	0.734
olfactory receptor, family 6, subfamily A, member 1	1.449 (1.004 to 2.173)	0.239
oligodendrocyte myelin glycoprotein	2.008 (1.591 to 2.59)	0.0386
oligophrenin 1	0.866 (0.672 to 1.231)	0.359
O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)	0.958 (0.956 to 0.96)	0.0263
oncomodulin	1.04 (0.484 to 1.775)	0.876
opioid receptor, delta 1	1.174 (1.132 to 1.218)	0.144
opioid receptor, kappa 1	0.732 (0.477 to 1.072)	0.315
opsin 1 (cone pigments), medium-wave-sensitive (color blindness, deutan)	1.19 (0.966 to 1.467)	0.558
opsin 1 (cone pigments), short-wave-sensitive (color blindness, tritan)	0.918 (0.606 to 1.39)	0.87
oral-facial-digital syndrome 1	0.873 (0.803 to 0.922)	0.0869
origin recognition complex, subunit 2-like (yeast)	0.935 (0.788 to 1.11)	0.763
origin recognition complex, subunit 4-like (yeast)	1.303 (0.693 to 2.293)	0.376
origin recognition complex, subunit 5-like (yeast)	0.502 (0.237 to 1.518)	0.347
ornithine aminotransferase (gyrate atrophy)	0.662 (0.189 to 1.34)	0.579

ornithine carbamoyltransferase	0.837 (0.321 to 1.554)	0.75
ornithine decarboxylase 1	1.157 (1.094 to 1.224)	0.234
osteomodulin	0.754 (0.378 to 1.507)	0.754
outer dense fiber of sperm tails 2	1.27 (0.834 to 1.935)	0.671
oviductal glycoprotein 1, 120kDa (mucin 9, oviductin)	0.954 (0.637 to 1.273)	0.842
ovo-like 1(Drosophila)	0.787 (0.717 to 0.925)	0.0986
oxidase (cytochrome c) assembly 1-like	0.625 (0.614 to 0.637)	0.0241
oxidised low density lipoprotein (lectin-like) receptor 1	1.093 (0.799 to 1.6)	0.583
oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)	1.002 (0.823 to 1.231)	0.985
oxysterol binding protein	0.835 (0.544 to 1.055)	0.491
oxytocin, prepro- (neurophysin I)	0.994 (0.588 to 1.28)	0.97
p21 (CDKN1A)-activated kinase 2	0.703 (0.506 to 1.047)	0.121
paired basic amino acid cleaving system 4	1.022 (0.637 to 1.339)	0.877
paired box gene 2	1.161 (1.13 to 1.192)	0.114
paired box gene 3 (Waardenburg syndrome 1)	0.946 (0.716 to 1.108)	0.572
paired box gene 4	0.986 (0.895 to 1.085)	0.82
paired box gene 6 (aniridia, keratitis)	0.904	no replicates
paired box gene 7	1.447 (1.097 to 2.482)	0.148
paired box gene 8	1.419 (1.396 to 1.441)	0.0289
paired-like (aristaless) homeobox 2a	1.442 (1.151 to 1.807)	0.352
paired-like homeodomain transcription factor 1	2.267 (1.566 to 3.282)	0.27
paired-like homeodomain transcription factor 2	0.963 (0.756 to 1.425)	0.766
paired-like homeodomain transcription factor 3	1.315 (0.84 to 1.965)	0.383
palmitoyl-protein thioesterase 1 (ceroid-lipofuscinosis, neuronal 1, infantile)	1.089 (1.07 to 1.107)	0.0126
pancreatic lipase-related protein 1	1.597 (1.151 to 3.222)	0.144
pancreatic lipase-related protein 2	1.362 (1.051 to 1.765)	0.445
pancreatic polypeptide	0.753 (0.561 to 1.042)	0.254
pancreatic polypeptide receptor 1	0.99 (0.934 to 1.049)	0.891
papillary renal cell carcinoma (translocation-associated)	1.304 (1.214 to 1.402)	0.169
paralemmin	0.642 (0.475 to 0.868)	0.38
paraoxonase 2	1.035 (0.966 to 1.11)	0.705
parathymosin	1.137 (0.982 to 1.496)	0.45
parathyroid hormone	1.773 (1.005 to 3.128)	0.497
parathyroid hormone receptor 1	1.09 (0.912 to 1.314)	0.502
parathyroid hormone receptor 2	0.728 (0.696 to 0.761)	0.0889
parvalbumin	0.927 (0.486 to 1.322)	0.756
patched homolog 2 (Drosophila)	1.132 (0.863 to 1.88)	0.674
PBX/knotted 1 homeobox 1	0.798 (0.344 to 1.243)	0.647
PDZ domain containing 1	2.145	no replicates
peanut-like 1 (Drosophila)	1.061 (0.849 to 1.536)	0.588
peanut-like 2 (Drosophila)	0.949 (0.558 to 1.614)	0.938
pentaxin-related gene, rapidly induced by IL-1 beta	0.932 (0.893 to 0.973)	0.347
peptidase D	0.86 (0.518 to 1.182)	0.462
peptide YY	1.052 (0.765 to 1.579)	0.835
peptidylglycine alpha-amidating monooxygenase	0.97 (0.859 to 1.062)	0.677
peptidylprolyl isomerase B (cyclophilin B)	2.268 (1.2 to 7.446)	0.303
peptidylprolyl isomerase C (cyclophilin C)	0.777 (0.501 to 1.466)	0.518
peptidylprolyl isomerase D (cyclophilin D)	1.415 (0.968 to 2.609)	0.377
perforin 1 (pore forming protein)	1.091 (0.574 to 2.593)	0.864
pericentrin 2 (kendrin)	1.222 (1.165 to 1.283)	0.15
pericentriolar material 1	0.825 (0.663 to 1.028)	0.149
perilipin	1.215 (0.523 to 2.078)	0.48
period homolog 1 (Drosophila)	1.071 (0.845 to 1.412)	0.597
peripheral myelin protein 2	1.11 (0.475 to 2.056)	0.756
peripherin	1.267	no replicates
periplakin	0.994 (0.927 to 1.186)	0.927
peroxiredoxin 1	1.162 (0.788 to 1.596)	0.395
peroxiredoxin 3	1.012 (0.996 to 1.029)	0.585
peroxiredoxin 4	1.064	no replicates
peroxisomal biogenesis factor 11B	1.044 (1.015 to 1.073)	0.364
peroxisomal biogenesis factor 12	0.727 (0.531 to 0.935)	0.196
peroxisomal biogenesis factor 14	1.303 (1.274 to 1.333)	0.0539
peroxisomal biogenesis factor 3	1.658 (1.047 to 4.243)	0.219
peroxisomal biogenesis factor 6	0.902 (0.519 to 1.475)	0.667
peroxisomal biogenesis factor 7	1.275 (0.995 to 1.958)	0.377
peroxisomal farnesylated protein	1.898 (0.903 to 7.916)	0.182
peroxisomal membrane protein 3, 35kDa (Zellweger syndrome)	1.127 (0.791 to 1.604)	0.793
peroxisome biogenesis factor 1	1.06 (0.979 to 1.147)	0.598
peroxisome biogenesis factor 10	1.601 (0.891 to 2.876)	0.569

peroxisome biogenesis factor 13	2.276 (2.267 to 2.286)	0.00331
peroxisome proliferative activated receptor, alpha	1.082 (0.442 to 2.082)	0.881
peroxisome proliferative activated receptor, delta	1.104 (0.907 to 1.83)	0.602
peroxisome proliferative activated receptor, gamma	0.909 (0.685 to 1.079)	0.411
peroxisome receptor 1	1.051 (0.529 to 2.196)	0.915
persephin	1.071 (0.986 to 1.316)	0.396
PET112-like (yeast)	1.037 (0.768 to 1.422)	0.858
PHD finger protein 2	0.914 (0.565 to 1.295)	0.751
phenylalanine-tRNA synthetase-like	1.518 (1.184 to 2.103)	0.134
phosducin	0.839 (0.347 to 2.494)	0.61
phosducin-like	1.155 (0.884 to 1.742)	0.561
phosphate cytidylyltransferase 1, choline, alpha isoform	1.958 (1.314 to 2.918)	0.341
phosphatidic acid phosphatase type 2A	1.624 (1.383 to 1.908)	0.204
phosphatidic acid phosphatase type 2B	0.935 (0.894 to 0.978)	0.373
phosphatidic acid phosphatase type 2C	0.4	no replicates
phosphatidylinositol 4-kinase, catalytic, beta polypeptide	0.987 (0.906 to 1.076)	0.904
phosphatidylinositol glycan, class H	1.481 (0.911 to 1.962)	0.249
phosphatidylinositol-4-phosphate 5-kinase, type I, beta	1.026 (0.866 to 1.215)	0.904
phosphatidylinositol-4-phosphate 5-kinase, type II, alpha	1.003 (0.575 to 1.864)	0.989
phosphodiesterase 1C, calmodulin-dependent 70kDa	1.35 (1.019 to 1.787)	0.479
phosphodiesterase 3B, cGMP-inhibited	1.054 (0.894 to 1.189)	0.598
phosphodiesterase 4A, cAMP-specific (phosphodiesterase E2 dunce homolog, Drosophila)	0.982 (0.873 to 1.266)	0.843
phosphodiesterase 4C, cAMP-specific (phosphodiesterase E1 dunce homolog, Drosophila)	1.536 (1.313 to 1.797)	0.223
phosphodiesterase 5A, cGMP-specific	0.785 (0.57 to 1.338)	0.462
phosphodiesterase 6B, cGMP-specific, rod, beta (congenital stationary night blindness 3, autosomal dominant)	1.014 (0.791 to 1.27)	0.915
phosphodiesterase 6C, cGMP-specific, cone, alpha prime	0.862 (0.493 to 1.316)	0.556
phosphodiesterase 6D, cGMP-specific, rod, delta	1.304 (0.614 to 3.771)	0.431
phosphodiesterase 6G, cGMP-specific, rod, gamma	0.989 (0.89 to 1.099)	0.933
phosphodiesterase 6H, cGMP-specific, cone, gamma	1.332 (0.857 to 1.786)	0.164
phosphodiesterase 9A	1.088 (1.027 to 1.154)	0.385
phosphoenolpyruvate carboxykinase 1 (soluble)	0.6 (0.0943 to 1.527)	0.474
phosphoenolpyruvate carboxykinase 2 (mitochondrial)	1.379 (1.042 to 1.826)	0.457
phosphofructokinase, liver	1.046 (0.743 to 1.763)	0.834
phosphofructokinase, muscle	1.034 (0.766 to 1.39)	0.801
phosphoglucomutase 1	1.222 (0.923 to 2.361)	0.313
phosphogluconate dehydrogenase	0.621 (0.489 to 0.81)	0.0826
phosphoglycerate kinase 1	1.141	no replicates
phosphoglycerate mutase 1 (brain)	1.363 (1.106 to 1.68)	0.378
phosphoglycerate mutase 2 (muscle)	1.113 (0.625 to 2.156)	0.794
phosphoinositide-3-kinase, catalytic, beta polypeptide	1.036 (0.713 to 1.728)	0.883
phosphoinositide-3-kinase, catalytic, delta polypeptide	1.131 (1.016 to 1.26)	0.458
phosphoinositide-3-kinase, catalytic, gamma polypeptide	0.96 (0.85 to 1.046)	0.583
phosphoinositide-3-kinase, class 2, alpha polypeptide	0.844 (0.669 to 1.065)	0.599
phosphoinositide-3-kinase, class 3	1.386 (1.221 to 1.573)	0.235
phosphoinositide-3-kinase, regulatory subunit, polypeptide 2 (p85 beta)	1.079 (0.681 to 1.483)	0.584
phosphoinositide-3-kinase, regulatory subunit, polypeptide 3 (p55, gamma)	1.18 (0.944 to 1.595)	0.4
phospholamban	0.981 (0.788 to 1.162)	0.849
phospholipase A2, group IB (pancreas)	0.893 (0.86 to 0.964)	0.0981
phospholipase A2, group IIA (platelets, synovial fluid)	0.782 (0.638 to 0.96)	0.443
phospholipase A2, group IVC (cytosolic, calcium-independent)	1.02 (0.302 to 1.7)	0.952
phospholipase A2, group V	1.589 (0.842 to 2.726)	0.157
phospholipase A2, group X	0.89	no replicates
phospholipase C, beta 4	0.776 (0.603 to 0.998)	0.498
phospholipase D2	1.337 (1.213 to 1.473)	0.206
phospholipid transfer protein	1.169 (1.13 to 1.215)	0.0175
phosphomannomutase 1	0.889 (0.808 to 0.937)	0.133
phosphomannomutase 2	0.806 (0.757 to 0.857)	0.176
phosphoprotein enriched in astrocytes 15	1.003 (0.858 to 1.173)	0.986
phosphoribosyl pyrophosphate amidotransferase	1.051 (1.025 to 1.078)	0.298
phosphoribosyl pyrophosphate synthetase 2	1.031 (0.55 to 1.864)	0.938
phosphoribosyl pyrophosphate synthetase-associated protein 1	0.928 (0.692 to 1.108)	0.449
phosphoribosyl pyrophosphate synthetase-associated protein 2	1.154 (0.746 to 1.621)	0.441
phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase	1.355 (1 to 2.017)	0.163
phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	0.839 (0.663 to 1.221)	0.452

phosphorylase kinase, alpha 1 (muscle)	1.881	no replicates
phosphorylase kinase, alpha 2 (liver)	0.906 (0.718 to 1.23)	0.601
phosphorylase kinase, beta	1.141 (1.029 to 1.236)	0.135
phosphorylase kinase, gamma 1 (muscle)	1.06 (0.768 to 1.956)	0.74
phosphorylase, glycogen; brain	1.08 (0.873 to 1.585)	0.547
phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI)	0.889 (0.822 to 0.962)	0.377
phosphorylase, glycogen; muscle (McArdle syndrome, glycogen storage disease type V)	1.014 (0.831 to 1.236)	0.957
phosphoserine phosphatase	0.949 (0.894 to 1.008)	0.546
phosphoserine phosphatase-like	0.827 (0.772 to 0.941)	0.098
phytanoyl-CoA hydroxylase (Refsum disease)	1.188 (1 to 1.368)	0.111
pim-1 oncogene	1.032 (0.522 to 1.427)	0.9
pinin, desmosome associated protein	1.193 (0.694 to 3.484)	0.661
Pirin	0.905 (0.868 to 0.944)	0.255
plakophilin 1 (ectodermal dysplasia/skin fragility syndrome)	0.759 (0.707 to 0.816)	0.162
plakophilin 2	1.323 (1.177 to 1.645)	0.125
plakophilin 4	0.831 (0.767 to 0.9)	0.259
plasminogen-like	1.27 (1.131 to 1.424)	0.0695
plastin 1 (I isoform)	1.117 (0.953 to 1.298)	0.341
plastin 3 (T isoform)	1.067 (0.854 to 1.333)	0.819
platelet factor 4 (chemokine (C-X-C motif) ligand 4)	0.896 (0.727 to 0.999)	0.402
platelet factor 4 variant 1	1.073 (0.977 to 1.14)	0.138
platelet/endothelial cell adhesion molecule (CD31 antigen)	0.953 (0.718 to 1.264)	0.892
platelet-activating factor acetylhydrolase 2, 40kDa	0.894 (0.829 to 1.059)	0.15
platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa	0.566	no replicates
platelet-activating factor acetylhydrolase, isoform Ib, beta subunit 30kDa	1.036 (0.677 to 1.384)	0.886
platelet-activating factor acetylhydrolase, isoform Ib, gamma subunit 29kDa	1.068 (0.97 to 1.177)	0.617
platelet-derived growth factor receptor-like	0.969 (0.909 to 1.033)	0.711
pleckstrin	0.934 (0.8 to 1.135)	0.574
pleckstrin and Sec7 domain protein	1.467 (1.18 to 1.774)	0.0836
plectin 1, intermediate filament binding protein 500kDa	1.457 (1.326 to 1.601)	0.156
pleiomorphic adenoma gene 1	1.009 (0.736 to 1.28)	0.925
pleiomorphic adenoma gene-like 1	0.829 (0.704 to 0.977)	0.458
pleiomorphic adenoma gene-like 2	0.976 (0.794 to 1.108)	0.839
pleiotropic regulator 1 (PRL1homolog, Arabidopsis)	1.189 (1.006 to 1.405)	0.489
plexin B1	1.349 (1.23 to 1.481)	0.191
podocalyxin-like	0.898 (0.826 to 0.975)	0.417
poliovirus receptor	1.244 (0.831 to 1.862)	0.685
poliovirus receptor-related 2 (herpesvirus entry mediator B)	1.04 (0.649 to 1.674)	0.893
poly (ADP-ribose) glycohydrolase	1.093 (0.847 to 1.372)	0.59
poly(A) binding protein, cytoplasmic 1	1.4 (0.803 to 2.44)	0.654
poly(A) binding protein, cytoplasmic 4 (inducible form)	1.06 (0.702 to 1.601)	0.91
poly(A) binding protein, nuclear 1	0.853 (0.722 to 1.008)	0.515
poly(A)-specific ribonuclease (deadenylation nuclease)	0.693 (0.272 to 0.958)	0.323
poly(rC) binding protein 1	1.496 (1.099 to 2.038)	0.416
poly(rC) binding protein 2	1.042 (0.854 to 1.273)	0.87
polycystic kidney disease 2 (autosomal dominant)	1.213 (1.155 to 1.274)	0.158
polyhomeotic-like 2 (Drosophila)	0.888 (0.466 to 2.413)	0.686
polymerase (DNA directed), delta 2, regulatory subunit 50kDa	1.192 (0.671 to 1.796)	0.613
polymerase (DNA directed), epsilon 2 (p59 subunit)	1.105 (0.885 to 1.591)	0.407
polymerase (DNA directed), eta	1.035 (0.856 to 1.383)	0.717
polymerase (RNA) II (DNA directed) polypeptide A, 220kDa	1.221 (1.142 to 1.305)	0.206
polymerase (RNA) II (DNA directed) polypeptide B, 140kDa	1.157 (1.045 to 1.281)	0.388
polymerase (RNA) II (DNA directed) polypeptide C, 33kDa	1.01 (0.874 to 1.196)	0.885
polymerase (RNA) II (DNA directed) polypeptide D	0.932 (0.503 to 1.726)	0.927
polymerase (RNA) II (DNA directed) polypeptide E, 25kDa	0.832 (0.565 to 1.422)	0.575
polymerase (RNA) II (DNA directed) polypeptide G	0.782 (0.522 to 1.161)	0.347
polymerase (RNA) II (DNA directed) polypeptide I, 14.5kDa	0.817 (0.605 to 1.102)	0.622
polymerase (RNA) II (DNA directed) polypeptide J, 13.3kDa	0.989 (0.853 to 1.148)	0.954
polymerase (RNA) II (DNA directed) polypeptide K, 7.0kDa	1.637 (1.079 to 2.483)	0.447
polymerase (RNA) III (DNA directed) polypeptide D, 44kDa	0.699 (0.167 to 1.7)	0.67
polymerase (RNA) mitochondrial (DNA directed)	1.145 (0.746 to 1.548)	0.466
polymyositis/scleroderma autoantigen 1, 75kDa	1.956 (1.331 to 2.874)	0.331
polypyrimidine tract binding protein 1	1.594 (1.457 to 1.745)	0.122
postmeiotic segregation increased 2-like 11	0.96 (0.956 to 0.963)	0.0568
postmeiotic segregation increased 2-like 8	1.302 (1.146 to 1.478)	0.286
potassium channel, subfamily K, member 1	0.907 (0.625 to 1.123)	0.381
potassium channel, subfamily K, member 3	1.038 (0.846 to 1.275)	0.884

potassium channel, subfamily K, member 5	0.542	no replicates
potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3	0.825	no replicates
potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	0.88 (0.331 to 1.656)	0.654
potassium inwardly-rectifying channel, subfamily J, inhibitor 1	1.219 (1.218 to 1.219)	0.000159
potassium inwardly-rectifying channel, subfamily J, member 10	0.943 (0.544 to 1.536)	0.757
potassium inwardly-rectifying channel, subfamily J, member 15	0.851 (0.253 to 1.947)	0.734
potassium inwardly-rectifying channel, subfamily J, member 2	0.874 (0.732 to 1.007)	0.29
potassium inwardly-rectifying channel, subfamily J, member 4	1.472 (0.737 to 4.754)	0.417
potassium inwardly-rectifying channel, subfamily J, member 5	1.123 (0.875 to 1.755)	0.657
potassium large conductance calcium-activated channel, subfamily M, alpha member 1	1.429 (1.03 to 1.985)	0.473
potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3	1.195 (0.887 to 1.61)	0.658
potassium voltage-gated channel, Isk-related family, member 1	1.369 (1.109 to 1.691)	0.00959
potassium voltage-gated channel, KQT-like subfamily, member 1	1.142 (0.726 to 1.846)	0.591
potassium voltage-gated channel, KQT-like subfamily, member 3	0.666 (0.178 to 1.397)	0.602
potassium voltage-gated channel, Shab-related subfamily, member 1	0.981 (0.893 to 1.056)	0.731
potassium voltage-gated channel, shaker-related subfamily, beta member 1	1.417 (1.261 to 1.593)	0.206
potassium voltage-gated channel, shaker-related subfamily, beta member 2	1.25 (0.83 to 3.667)	0.579
potassium voltage-gated channel, shaker-related subfamily, member 10	1.014 (0.823 to 1.374)	0.935
potassium voltage-gated channel, shaker-related subfamily, member 3	1.154 (1.022 to 1.303)	0.448
potassium voltage-gated channel, shaker-related subfamily, member 4	0.963 (0.751 to 1.126)	0.791
potassium voltage-gated channel, shaker-related subfamily, member 5	1.07 (0.651 to 1.44)	0.811
potassium voltage-gated channel, shaker-related subfamily, member 6	1.626 (1 to 2.644)	0.5
potassium voltage-gated channel, Shal-related subfamily, member 1	0.909 (0.576 to 1.211)	0.479
potassium voltage-gated channel, Shal-related subfamily, member 3	1.112 (0.599 to 2.5)	0.7
potassium voltage-gated channel, Shaw-related subfamily, member 1	0.871 (0.522 to 1.548)	0.578
potassium voltage-gated channel, Shaw-related subfamily, member 3	0.762 (0.486 to 1.212)	0.283
potassium voltage-gated channel, Shaw-related subfamily, member 4	1.219 (0.904 to 1.891)	0.249
potassium voltage-gated channel, subfamily F, member 1	1.078 (0.941 to 1.235)	0.677
potassium voltage-gated channel, subfamily G, member 1	1.2 (0.905 to 1.445)	0.199
potassium voltage-gated channel, subfamily H (eag-related), member 1	1.136 (0.623 to 1.981)	0.547
potassium voltage-gated channel, subfamily H (eag-related), member 2	1.198 (1.078 to 1.322)	0.0918
POU domain, class 1, transcription factor 1 (Pit1, growth hormone factor 1)	0.919 (0.793 to 1.064)	0.666
POU domain, class 2, associating factor 1	0.83 (0.535 to 1.474)	0.345
POU domain, class 3, transcription factor 1	1.252 (1.196 to 1.31)	0.127
POU domain, class 3, transcription factor 2	1.136 (0.891 to 1.337)	0.411
POU domain, class 3, transcription factor 3	1.79 (1.273 to 2.517)	0.337
POU domain, class 3, transcription factor 4	1.165 (1.117 to 1.216)	0.173
POU domain, class 4, transcription factor 1	1.443 (1.06 to 1.889)	0.161
POU domain, class 4, transcription factor 2	0.947 (0.364 to 1.431)	0.84
POU domain, class 4, transcription factor 3	1.105 (0.952 to 1.221)	0.317
POU domain, class 6, transcription factor 1	0.893 (0.606 to 1.13)	0.465
PPAR binding protein	1.534 (0.773 to 3.726)	0.455
pre-B-cell leukemia transcription factor 2	0.966 (0.696 to 1.184)	0.853
pre-B-cell leukemia transcription factor 3	0.97 (0.882 to 1.068)	0.806
prefoldin 1	1.091 (1.052 to 1.133)	0.256
prefoldin 4	1.107 (1.054 to 1.162)	0.285
pregnancy specific beta-1-glycoprotein 1	0.889 (0.489 to 1.44)	0.532
pregnancy specific beta-1-glycoprotein 11	1.155 (1.108 to 1.205)	0.181
pregnancy specific beta-1-glycoprotein 5	1.099 (1.04 to 1.161)	0.338
pregnancy specific beta-1-glycoprotein 7	1.88 (1.774 to 1.993)	0.0585
pregnancy specific beta-1-glycoprotein 9	1.489 (0.375 to 4.831)	0.42
pregnancy-associated plasma protein A	1.055 (0.843 to 1.432)	0.769
pregnancy-zone protein	1.006 (1.005 to 1.006)	0.0461
presenilin 1 (Alzheimer disease 3)	0.963 (0.642 to 1.823)	0.845
presenilin 2 (Alzheimer disease 4)	1.387 (1.252 to 1.537)	0.193
pre-T/NK cell associated protein	1.104 (0.858 to 1.786)	0.721
primase, polypeptide 1, 49kDa	1.026 (0.798 to 1.199)	0.861
primase, polypeptide 2A, 58kDa	1.511 (0.956 to 3.363)	0.412
prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia)	0.539 (0.48 to 0.577)	0.00875
PRKC, apoptosis, WT1, regulator	0.73 (0.721 to 0.739)	0.0251
procollagen (type III) N-endopeptidase	0.808 (0.505 to 1.05)	0.459
procollagen C-endopeptidase enhancer	1.398 (1.064 to 1.727)	0.144
procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2	1.563 (1.556 to 1.57)	0.00657

procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase, Ehlers-Danlos syndrome type VI)	0.962 (0.424 to 2.236)	0.918
procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I	1.452 (1.151 to 2.175)	0.208
procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide isomerase; thyroid hormone binding protein p55)	0.822 (0.805 to 0.839)	0.0667
profilin 1	0.958 (0.93 to 0.986)	0.377
profilin 2	1.257 (0.895 to 3.059)	0.498
progastricsin (pepsinogen C)	1.162 (0.798 to 1.802)	0.592
progestagen-associated endometrial protein (placental protein 14, pregnancy-associated endometrial alpha-2-globulin, alpha uterine protein)	1.086 (1.004 to 1.244)	0.348
progesterone receptor	1.29 (0.689 to 2.167)	0.283
programmed cell death 1	1.123 (0.869 to 1.45)	0.73
prolactin	0.959	no replicates
prolactin receptor	1.257 (0.915 to 1.974)	0.268
prolactin-induced protein	0.768 (0.591 to 1.031)	0.137
proliferating cell nuclear antigen	0.701 (0.431 to 1.14)	0.598
proliferation-associated 2G4, 38kDa	0.79 (0.316 to 1.448)	0.521
proline arginine-rich end leucine-rich repeat protein	0.815 (0.771 to 0.863)	0.172
proline rich 2	1.138 (0.604 to 1.936)	0.74
proline-rich Gla (G-carboxyglutamic acid) polypeptide 1	1.405 (1.147 to 1.772)	0.115
proline-rich Gla (G-carboxyglutamic acid) polypeptide 2	0.895 (0.269 to 1.847)	0.872
proline-rich protein BstNI subfamily 4	1.159 (0.881 to 1.429)	0.411
proline-rich protein HaeIII subfamily 2	1.215 (0.905 to 1.714)	0.166
prolyl endopeptidase	1.202 (1.064 to 1.294)	0.0954
prolylcarboxypeptidase (angiotensinase C)	0.963 (0.704 to 1.197)	0.779
pro-melanin-concentrating hormone	0.996 (0.735 to 1.258)	0.98
promyelocytic leukemia	1.01 (0.95 to 1.073)	0.902
proopiomelanocortin (adrenocorticotropin/ beta-lipotropin/ alpha-melanocyte stimulating hormone/ beta-melanocyte stimulating hormone/ beta-endorphin)	1.243 (1.074 to 1.44)	0.377
properdin P factor, complement	1.132 (0.713 to 1.454)	0.389
prophet of Pit1, paired-like homeodomain transcription factor	1.004 (0.964 to 1.046)	0.932
propionyl Coenzyme A carboxylase, alpha polypeptide	0.727 (0.572 to 0.924)	0.411
propionyl Coenzyme A carboxylase, beta polypeptide	0.885 (0.787 to 0.942)	0.0564
prospero-related homeobox 1	1.252 (0.66 to 1.994)	0.403
prostaglandin D2 synthase 21kDa (brain)	1.18 (0.968 to 1.438)	0.557
prostaglandin E receptor 1 (subtype EP1), 42kDa	0.905 (0.868 to 0.944)	0.252
prostaglandin E receptor 2 (subtype EP2), 53kDa	1.256 (1.049 to 1.374)	0.127
prostaglandin F receptor (FP)	0.771 (0.289 to 1.437)	0.653
prostaglandin I2 (prostacyclin) receptor (IP)	1.095 (0.942 to 1.387)	0.529
prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	1.476 (0.837 to 2.601)	0.617
prostate cancer overexpressed gene 1	0.988 (0.857 to 1.217)	0.921
prostatic binding protein	0.793 (0.326 to 1.991)	0.576
protamine 1	1.258 (1.076 to 1.471)	0.38
protamine 2	1.193 (0.855 to 1.664)	0.69
protease inhibitor 3, skin-derived (SKALP)	0.9 (0.883 to 0.918)	0.115
protease, serine, 1 (trypsin 1)	1.094 (0.867 to 1.379)	0.766
protease, serine, 11 (IGF binding)	1.601 (1.029 to 2.929)	0.271
protease, serine, 12 (neurotrypsin, motopsin)	1.394 (1 to 2.566)	0.39
protease, serine, 2 (trypsin 2)	2.264 (1.622 to 3.755)	0.0864
protease, serine, 3 (mesotrypsin)	0.911 (0.61 to 1.267)	0.706
protease, serine, 8 (prostasin)	0.859 (0.778 to 0.949)	0.37
proteasome (prosome, macropain) 26S subunit, ATPase, 1	1.108 (0.84 to 1.56)	0.568
proteasome (prosome, macropain) 26S subunit, ATPase, 2	0.847 (0.388 to 1.087)	0.447
proteasome (prosome, macropain) 26S subunit, ATPase, 4	1.231 (1.054 to 1.437)	0.409
proteasome (prosome, macropain) 26S subunit, ATPase, 5	2.555	no replicates
proteasome (prosome, macropain) 26S subunit, ATPase, 6	1.242 (1.079 to 1.43)	0.366
proteasome (prosome, macropain) 26S subunit, non-ATPase, 1	0.963 (0.684 to 1.411)	0.873
proteasome (prosome, macropain) 26S subunit, non-ATPase, 10	0.964 (0.614 to 1.35)	0.891
proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	1.366 (0.874 to 1.864)	0.179
proteasome (prosome, macropain) 26S subunit, non-ATPase, 12	1.024 (0.907 to 1.179)	0.782
proteasome (prosome, macropain) 26S subunit, non-ATPase, 13	0.633 (0.382 to 0.883)	0.216
proteasome (prosome, macropain) 26S subunit, non-ATPase, 2	1.013 (0.964 to 1.065)	0.838
proteasome (prosome, macropain) 26S subunit, non-ATPase, 3	1.172 (0.985 to 1.395)	0.529
proteasome (prosome, macropain) 26S subunit, non-ATPase, 4	1.119 (0.895 to 1.4)	0.703
proteasome (prosome, macropain) 26S subunit, non-ATPase, 5	1.189 (0.947 to 1.414)	0.282
proteasome (prosome, macropain) 26S subunit, non-ATPase, 7 (Mov34	0.978 (0.782 to 1.222)	0.935

homolog)		
proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	1.182 (0.808 to 1.59)	0.242
proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	0.881 (0.469 to 1.625)	0.744
proteasome (prosome, macropain) activator subunit 2 (PA28 beta)	1.795 (1.011 to 3.925)	0.0786
proteasome (prosome, macropain) subunit, alpha type, 5	0.793 (0.182 to 2.686)	0.797
proteasome (prosome, macropain) subunit, alpha type, 7	1.148 (0.908 to 1.305)	0.103
proteasome (prosome, macropain) subunit, beta type, 1	1 (0.84 to 1.45)	0.998
proteasome (prosome, macropain) subunit, beta type, 10	1.45 (0.939 to 2.24)	0.55
proteasome (prosome, macropain) subunit, beta type, 2	0.52 (0.123 to 1.309)	0.464
proteasome (prosome, macropain) subunit, beta type, 3	1.181 (1.064 to 1.311)	0.356
proteasome (prosome, macropain) subunit, beta type, 4	1.057 (0.796 to 1.404)	0.876
proteasome (prosome, macropain) subunit, beta type, 5	0.859 (0.685 to 1.14)	0.417
proteasome (prosome, macropain) subunit, beta type, 7	1.032 (0.616 to 1.536)	0.917
proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional protease 7)	1.509 (1.287 to 1.77)	0.235
protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1-like	1.551 (0.911 to 5.724)	0.258
protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)	0.628 (0.595 to 0.685)	0.0086
protein C (inactivator of coagulation factors Va and VIIIa)	1.032 (0.526 to 2.257)	0.91
protein geranylgeranyltransferase type I, beta subunit	1.652 (1.008 to 2.708)	0.495
protein inhibitor of activated STAT, 1	0.595 (0.231 to 1.535)	0.681
protein kinase C, alpha	1.158 (0.814 to 2.1)	0.554
protein kinase C, delta	0.819 (0.38 to 1.258)	0.513
protein kinase C, iota	0.573 (0.376 to 0.871)	0.411
protein kinase C, mu	1.351 (0.963 to 2.255)	0.367
protein kinase C, theta	0.965 (0.86 to 1.121)	0.693
protein kinase C-like 1	0.886 (0.626 to 1.56)	0.713
protein kinase C-like 2	1.053 (0.794 to 1.532)	0.65
protein kinase, AMP-activated, alpha 2 catalytic subunit	1.231 (1.032 to 1.658)	0.137
protein kinase, AMP-activated, beta 1 non-catalytic subunit	1.124 (1.086 to 1.164)	0.182
protein kinase, AMP-activated, beta 2 non-catalytic subunit	0.876	no replicates
protein kinase, AMP-activated, gamma 1 non-catalytic subunit	1.486 (1.416 to 1.559)	0.0768
protein kinase, cAMP-dependent, catalytic, gamma	1.785 (0.93 to 5.195)	0.395
protein kinase, cGMP-dependent, type II	0.775 (0.606 to 0.989)	0.486
protein kinase, interferon-inducible double stranded RNA dependent activator	0.825 (0.589 to 1.161)	0.43
protein kinase, X-linked	1.389 (1.033 to 1.868)	0.467
protein phosphatase 1, catalytic subunit, beta isoform	0.969 (0.96 to 0.978)	0.185
protein phosphatase 1, catalytic subunit, gamma isoform	0.807 (0.71 to 0.904)	0.0929
protein phosphatase 1, regulatory (inhibitor) subunit 12A	1.462 (1.414 to 1.512)	0.0557
protein phosphatase 1, regulatory (inhibitor) subunit 12B	1.336 (1.131 to 1.871)	0.0885
protein phosphatase 1, regulatory (inhibitor) subunit 1A	0.936 (0.67 to 1.167)	0.735
protein phosphatase 1, regulatory (inhibitor) subunit 2	1.242 (1.106 to 1.394)	0.312
protein phosphatase 1, regulatory (inhibitor) subunit 3A (glycogen and sarcoplasmic reticulum binding subunit, skeletal muscle)	1.124 (0.878 to 1.232)	0.136
protein phosphatase 1, regulatory (inhibitor) subunit 3C	0.938 (0.888 to 0.992)	0.455
protein phosphatase 1, regulatory (inhibitor) subunit 8	0.97 (0.824 to 1.141)	0.881
protein phosphatase 1, regulatory subunit 10	0.987 (0.881 to 1.091)	0.853
protein phosphatase 1, regulatory subunit 3D	0.848 (0.487 to 1.28)	0.482
protein phosphatase 1, regulatory subunit 7	0.956 (0.938 to 0.974)	0.25
protein phosphatase 1B (formerly 2C), magnesium-dependent, beta isoform	0.999 (0.755 to 1.692)	0.995
protein phosphatase 1D magnesium-dependent, delta isoform	0.448 (0.0816 to 1.158)	0.446
protein phosphatase 1G (formerly 2C), magnesium-dependent, gamma isoform	1 (0.778 to 1.286)	1
protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform	1.097 (0.925 to 1.419)	0.554
protein phosphatase 2 (formerly 2A), regulatory subunit B", alpha	0.81 (0.532 to 1.064)	0.259
protein phosphatase 2, regulatory subunit B (B56), alpha isoform	0.849 (0.808 to 0.891)	0.185
protein phosphatase 2, regulatory subunit B (B56), beta isoform	1.329 (0.993 to 1.779)	0.508
protein phosphatase 2, regulatory subunit B (B56), delta isoform	0.411 (0.319 to 0.53)	0.177
protein phosphatase 2, regulatory subunit B (B56), epsilon isoform	1.085 (0.784 to 1.431)	0.688
protein phosphatase 2, regulatory subunit B (B56), gamma isoform	0.818 (0.763 to 0.878)	0.216
protein phosphatase 3 (formerly 2B), catalytic subunit, gamma isoform (calcineurin A gamma)	1.029 (0.647 to 1.832)	0.934
protein phosphatase 4 (formerly X), catalytic subunit	0.95 (0.865 to 1.051)	0.46
protein phosphatase 5, catalytic subunit	1.092 (0.85 to 1.404)	0.607
protein phosphatase 6, catalytic subunit	1.106 (1.01 to 1.21)	0.466
protein phosphatase, EF hand calcium-binding domain 1	1.249 (0.609 to 4.039)	0.518
protein S (alpha)	0.65 (0.567 to 0.745)	0.195
protein tyrosine phosphatase type IVA, member 2	1.397 (0.884 to 2.604)	0.409
protein tyrosine phosphatase, non-receptor type 12	0.973 (0.668 to 1.213)	0.853
protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-	0.893 (0.855 to 0.933)	0.235

associated phosphatase)		
protein tyrosine phosphatase, non-receptor type 14	1.092 (0.867 to 1.491)	0.409
protein tyrosine phosphatase, non-receptor type 2	1.036 (0.506 to 3.328)	0.958
protein tyrosine phosphatase, non-receptor type 3	1.238 (1.038 to 1.705)	0.313
protein tyrosine phosphatase, non-receptor type 4 (megakaryocyte)	1.371 (0.957 to 2.355)	0.213
protein tyrosine phosphatase, non-receptor type 6	1.404 (0.97 to 2.836)	0.163
protein tyrosine phosphatase, non-receptor type 9	0.956 (0.708 to 1.513)	0.805
protein tyrosine phosphatase, non-receptor type substrate 1	1.048 (0.879 to 1.543)	0.744
protein tyrosine phosphatase, receptor type, A	1.502	no replicates
protein tyrosine phosphatase, receptor type, B	1.132 (1.081 to 1.185)	0.224
protein tyrosine phosphatase, receptor type, C-associated protein	0.743 (0.446 to 1.003)	0.365
protein tyrosine phosphatase, receptor type, D	0.726 (0.592 to 0.96)	0.0529
protein tyrosine phosphatase, receptor type, E	0.707 (0.604 to 0.829)	0.274
protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 1	1.021 (0.858 to 1.216)	0.924
protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 2	1.19 (0.822 to 1.541)	0.215
protein tyrosine phosphatase, receptor type, J	0.952 (0.65 to 1.331)	0.776
protein tyrosine phosphatase, receptor type, K	3.798	no replicates
protein tyrosine phosphatase, receptor type, M	1.004 (0.865 to 1.36)	0.972
protein tyrosine phosphatase, receptor type, N	1.124 (0.652 to 1.613)	0.588
protein tyrosine phosphatase, receptor type, O	0.944 (0.635 to 1.214)	0.729
protein tyrosine phosphatase, receptor type, R	0.837 (0.442 to 1.258)	0.639
protein tyrosine phosphatase, receptor type, S	1.205 (0.723 to 2.665)	0.486
protein-kinase, interferon-inducible double stranded RNA dependent inhibitor, repressor of (P58 repressor)	0.819 (0.771 to 0.868)	0.0286
protein-L-isoaspartate (D-aspartate) O-methyltransferase	1.051 (0.954 to 1.157)	0.699
proteoglycan 1, secretory granule	0.711	no replicates
proteolipid protein 2 (colonic epithelium-enriched)	0.842 (0.476 to 1.361)	0.485
protocadherin 8	1.164 (1.158 to 1.17)	0.0215
protocadherin gamma subfamily C, 3	1.177 (0.843 to 1.507)	0.446
protoporphyrinogen oxidase	2.461 (1.47 to 4.119)	0.331
PRP18 pre-mRNA processing factor 18 homolog (yeast)	0.731 (0.376 to 1.341)	0.485
PTK2B protein tyrosine kinase 2 beta	0.719 (0.692 to 0.738)	0.00362
PTK6 protein tyrosine kinase 6	0.961 (0.93 to 0.992)	0.434
PTK9 protein tyrosine kinase 9	0.865 (0.819 to 0.914)	0.229
PTPRF interacting protein, binding protein 1 (liprin beta 1)	1.029 (0.93 to 1.215)	0.763
purinergic receptor P2X, ligand-gated ion channel, 4	0.95 (0.487 to 2.262)	0.85
purinergic receptor P2X, ligand-gated ion channel, 5	1.044 (0.962 to 1.111)	0.419
purinergic receptor P2X, ligand-gated ion channel, 7	0.998 (0.41 to 1.677)	0.993
purinergic receptor P2Y, G-protein coupled, 11	0.726 (0.386 to 1.465)	0.495
purinergic receptor P2Y, G-protein coupled, 2	1.341 (0.716 to 2.273)	0.476
putative tumor suppressor 101F6	1.536 (0.972 to 2.427)	0.52
PWP2 periodic tryptophan protein homolog (yeast)	0.713 (0.686 to 0.74)	0.0714
pyridoxal (pyridoxine, vitamin B6) kinase	1.279 (1.064 to 1.538)	0.409
pyrimidinergic receptor P2Y, G-protein coupled, 4	1.033 (1.026 to 1.04)	0.127
pyrimidinergic receptor P2Y, G-protein coupled, 6	1.007 (0.83 to 1.222)	0.977
pyrroline-5-carboxylate reductase 1	1.31 (1.198 to 1.408)	0.0059
pyrroline-5-carboxylate synthetase (glutamate gamma-semialdehyde synthetase)	0.979 (0.943 to 1.015)	0.661
pyruvate carboxylase	0.679 (0.559 to 0.823)	0.294
pyruvate dehydrogenase (lipoamide) alpha 1	1.357 (0.858 to 4.15)	0.474
pyruvate dehydrogenase (lipoamide) alpha 2	1.049 (0.988 to 1.113)	0.572
pyruvate dehydrogenase (lipoamide) beta	1.178 (0.93 to 1.492)	0.615
pyruvate dehydrogenase complex, component X	0.956 (0.785 to 1.166)	0.859
pyruvate dehydrogenase kinase, isoenzyme 2	1.146 (1.002 to 1.412)	0.198
pyruvate dehydrogenase kinase, isoenzyme 3	1.437 (1 to 2.074)	0.227
pyruvate dehydrogenase kinase, isoenzyme 4	1.175 (0.508 to 3.579)	0.729
pyruvate kinase, muscle	1.438 (1.33 to 1.638)	0.031
quinoid dihydropteridine reductase	1.08 (0.937 to 1.181)	0.396
Rab geranylgeranyltransferase, alpha subunit	1.098 (0.922 to 1.814)	0.617
Rab geranylgeranyltransferase, beta subunit	4.461	no replicates
RAB interacting factor	1.21 (0.937 to 2.256)	0.428
RAB13, member RAS oncogene family	0.924 (0.711 to 1.052)	0.446
RAB1A, member RAS oncogene family	1.238 (1.082 to 1.416)	0.358
RAB2, member RAS oncogene family	1.132 (0.944 to 1.357)	0.619
RAB2, member RAS oncogene family-like	1.08 (0.786 to 1.484)	0.849
RAB27A, member RAS oncogene family	0.791 (0.776 to 0.807)	0.0531
RAB27B, member RAS oncogene family	0.975 (0.793 to 1.198)	0.921
RAB3A, member RAS oncogene family	1.141 (0.856 to 1.66)	0.569

RAB4A, member RAS oncogene family	1.361 (0.973 to 2.039)	0.0737
RAB5B, member RAS oncogene family	0.493	no replicates
RAB5C, member RAS oncogene family	1.318 (0.557 to 2.779)	0.464
RAD1 homolog (S. pombe)	0.811 (0.599 to 1.151)	0.217
RAD17 homolog (S. pombe)	1.053 (0.781 to 1.794)	0.75
RAD21 homolog (S. pombe)	0.895	no replicates
RAD23 homolog A (S. cerevisiae)	0.996 (0.629 to 2.634)	0.99
RAD51 homolog C (S. cerevisiae)	1.056 (0.822 to 1.431)	0.771
RAD51-like 3 (S. cerevisiae)	1.271 (1.205 to 1.342)	0.14
RAD54-like (S. cerevisiae)	1.059 (1.03 to 1.09)	0.291
RAD9 homolog (S. pombe)	1.787 (1.602 to 1.992)	0.118
radixin	1.27 (1.095 to 1.393)	0.0858
RAE1 RNA export 1 homolog (S. pombe)	0.719 (0.246 to 1.355)	0.604
RAN binding protein 1	0.915 (0.851 to 0.984)	0.437
RAN binding protein 2	1.169 (0.935 to 1.475)	0.139
RAN binding protein 3	1.586 (1.144 to 2.843)	0.256
RAN, member RAS oncogene family	0.927 (0.725 to 1.438)	0.762
RAP1, GTPase activating protein 1	0.66 (0.626 to 0.696)	0.0801
RAP2B, member of RAS oncogene family	0.856 (0.752 to 0.975)	0.443
RAR-related orphan receptor B	1.012 (0.93 to 1.101)	0.912
RAR-related orphan receptor C	0.914 (0.838 to 0.997)	0.49
ras homolog gene family, member C	1.235 (1.006 to 1.515)	0.491
ras homolog gene family, member E	1.229 (1.207 to 1.252)	0.0565
ras homolog gene family, member G (rho G)	1.097 (0.503 to 2.149)	0.732
ras homolog gene family, member H	1.296 (1.197 to 1.402)	0.188
ras homolog gene family, member I	1.019 (0.795 to 1.445)	0.898
RAS p21 protein activator 2	1.147 (0.823 to 1.482)	0.417
RAS protein activator like 1 (GAP1 like)	0.548 (0.068 to 1.476)	0.339
Ras-homolog enriched in brain	1.252	no replicates
Ras-like without CAAX 1	1.306 (1.177 to 1.376)	0.0356
Ras-like without CAAX 2	0.558 (0.317 to 1.198)	0.173
ras-related C3 botulinum toxin substrate 3 (rho family, small GTP binding protein Rac3)	1.023 (0.968 to 1.133)	0.7
RD RNA binding protein	0.623 (0.351 to 0.881)	0.243
RE1-silencing transcription factor	1.028 (0.937 to 1.197)	0.756
receptor (TNFRSF)-interacting serine-threonine kinase 1	1.348 (1.003 to 2.307)	0.383
receptor tyrosine kinase-like orphan receptor 1	1.03 (0.813 to 1.305)	0.92
receptor tyrosine kinase-like orphan receptor 2	0.652 (0.604 to 0.705)	0.114
receptor-interacting serine-threonine kinase 2	2.948 (1.115 to 19.27)	0.369
reelin	1.037 (0.859 to 1.252)	0.879
regenerating islet-derived 1 alpha (pancreatic stone protein, pancreatic thread protein)	0.989 (0.979 to 0.999)	0.46
regulator of G-protein signalling 1	1.315 (1.241 to 1.393)	0.133
regulator of G-protein signalling 11	0.75 (0.436 to 1.284)	0.194
regulator of G-protein signalling 12	1.518 (1.186 to 1.943)	0.34
regulator of G-protein signalling 16	1.03 (1.019 to 1.045)	0.0586
regulator of G-protein signalling 2, 24kDa	0.979 (0.635 to 1.477)	0.938
regulator of G-protein signalling 20	1.112 (1.039 to 1.191)	0.362
regulator of G-protein signalling 5	1.585 (1 to 2.776)	0.138
regulator of G-protein signalling 9	0.901 (0.822 to 0.987)	0.457
regulator of nonsense transcripts 1	0.956 (0.817 to 1.117)	0.821
regulatory factor X, 1 (influences HLA class II expression)	0.86 (0.833 to 0.888)	0.135
regulatory factor X, 3 (influences HLA class II expression)	1.213	no replicates
regulatory factor X, 5 (influences HLA class II expression)	1.248 (0.754 to 3.985)	0.499
regulatory factor X-associated ankyrin-containing protein	1.133 (1.077 to 1.192)	0.245
regulatory factor X-associated protein	0.978 (0.819 to 1.139)	0.836
regulatory solute carrier protein, family 1, member 1	1.195 (1.009 to 1.336)	0.174
related RAS viral (r-ras) oncogene homolog	1.209 (1.067 to 1.37)	0.37
relaxin 1 (H1)	0.832 (0.428 to 1.247)	0.639
relaxin 2 (H2)	1.219 (0.571 to 2.353)	0.433
renin	0.683	no replicates
replication protein A2, 32kDa	1.058 (0.766 to 1.257)	0.759
requiem, apoptosis response zinc finger gene	1.191 (0.976 to 1.454)	0.542
restin (Reed-Steinberg cell-expressed intermediate filament-associated protein)	1.29 (1.049 to 1.586)	0.434
ret finger protein	1.229 (0.868 to 1.571)	0.369
reticulocalbin 1, EF-hand calcium binding domain	1.031 (0.429 to 1.813)	0.909
reticulocalbin 2, EF-hand calcium binding domain	1.069 (1.064 to 1.074)	0.0466
reticulon 2	0.964 (0.658 to 1.413)	0.939
retinal degeneration, slow	1.715 (0.655 to 4.492)	0.675

retinal G protein coupled receptor	0.744 (0.728 to 0.76)	0.0458
retinal outer segment membrane protein 1	0.641 (0.48 to 0.855)	0.366
retinal pigment epithelium-specific protein 65kDa	1.039 (0.794 to 1.803)	0.852
retinaldehyde binding protein 1	0.784 (0.503 to 1.371)	0.362
retinitis pigmentosa 1 (autosomal dominant)	1.112 (1.057 to 1.17)	0.283
retinitis pigmentosa 2 (X-linked recessive)	1.245 (0.999 to 1.552)	0.501
retinitis pigmentosa GTPase regulator	1.104 (1.011 to 1.205)	0.462
retinoblastoma binding protein 2	1.389 (1.204 to 1.602)	0.261
retinoblastoma binding protein 6	1.941	no replicates
retinoblastoma binding protein 8	1.346 (1.062 to 1.912)	0.106
retinoic acid receptor responder (tazarotene induced) 1	1.775 (1.335 to 2.545)	0.0945
retinoic acid receptor responder (tazarotene induced) 3	0.797 (0.258 to 1.469)	0.616
retinoic acid receptor, alpha	1.063 (0.655 to 1.85)	0.732
retinoid X receptor, gamma	1.049 (0.917 to 1.199)	0.783
retinol binding protein 1, cellular	0.692 (0.533 to 0.93)	0.0305
retinol binding protein 2, cellular	0.91 (0.835 to 0.992)	0.471
retinol binding protein 3, interstitial	1.055 (1 to 1.171)	0.413
retinol dehydrogenase 5 (11-cis and 9-cis)	1.446 (1.319 to 1.54)	0.0158
REV3-like, catalytic subunit of DNA polymerase zeta (yeast)	1.196 (1.062 to 1.29)	0.0963
Rhesus blood group-associated glycoprotein	1.258	no replicates
Rho GDP dissociation inhibitor (GDI) gamma	0.81 (0.571 to 1.149)	0.654
Rho GTPase activating protein 1	0.763 (0.488 to 1.194)	0.654
Rho GTPase activating protein 6	1.154 (1.015 to 1.311)	0.464
rhodopsin (opsin 2, rod pigment) (retinitis pigmentosa 4, autosomal dominant)	1.04 (0.968 to 1.117)	0.68
ribonuclease 6 precursor	1.338 (1.201 to 1.491)	0.227
ribonuclease H1	1.367 (1.322 to 1.414)	0.0681
ribonuclease, RNase A family, 1 (pancreatic)	0.967 (0.939 to 0.995)	0.453
ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin)	1.393 (1.14 to 1.701)	0.346
ribonuclease, RNase A family, 3 (eosinophil cationic protein)	1.045 (0.979 to 1.115)	0.624
ribonuclease, RNase A family, 4	1.279 (1.256 to 1.302)	0.0467
ribonuclease, RNase A family, k6	1.193	no replicates
ribonucleotide reductase M1 polypeptide	1.308 (0.687 to 2.353)	0.245
ribonucleotide reductase M2 polypeptide	1.129 (1.047 to 1.217)	0.354
ribophorin I	0.738 (0.515 to 1.057)	0.553
ribophorin II	4.38 (1.327 to 14.46)	0.433
ribosomal protein L10	1.135 (1.047 to 1.231)	0.363
ribosomal protein L10a	0.992 (0.641 to 1.535)	0.988
ribosomal protein L11	0.805 (0.778 to 0.834)	0.102
ribosomal protein L12	1.171 (0.891 to 1.488)	0.4
ribosomal protein L13	1.121 (0.947 to 1.851)	0.421
ribosomal protein L13a	0.787 (0.579 to 1.06)	0.304
ribosomal protein L15	1.147 (0.834 to 1.576)	0.742
ribosomal protein L17	1.154 (0.784 to 1.709)	0.413
ribosomal protein L18	0.968 (0.712 to 1.545)	0.815
ribosomal protein L19	1.744 (1.457 to 2.053)	0.00118
ribosomal protein L21	1.143 (1.013 to 1.288)	0.466
ribosomal protein L22	0.76 (0.541 to 1.016)	0.122
ribosomal protein L23a	1.101 (0.863 to 1.239)	0.326
ribosomal protein L24	1.34 (1.096 to 1.675)	0.0771
ribosomal protein L26	1.069 (1.058 to 1.08)	0.0968
ribosomal protein L27	1.146 (0.837 to 1.569)	0.74
ribosomal protein L27a	0.914 (0.803 to 1.206)	0.29
ribosomal protein L28	1.084 (0.769 to 1.543)	0.727
ribosomal protein L29	0.674 (0.47 to 0.967)	0.472
ribosomal protein L3	1.358 (0.693 to 1.896)	0.156
ribosomal protein L30	0.995 (0.816 to 1.32)	0.977
ribosomal protein L31	0.914 (0.341 to 1.535)	0.809
ribosomal protein L32	0.965 (0.923 to 1.009)	0.57
ribosomal protein L34	0.92 (0.679 to 1.116)	0.492
ribosomal protein L35a	1.147 (0.934 to 1.661)	0.258
ribosomal protein L36a-like	1.155 (0.689 to 2.393)	0.525
ribosomal protein L37	0.897 (0.702 to 1.077)	0.328
ribosomal protein L37a	0.955 (0.674 to 1.677)	0.844
ribosomal protein L38	0.911 (0.612 to 1.356)	0.853
ribosomal protein L39	0.669 (0.218 to 1.018)	0.361
ribosomal protein L3-like	0.815 (0.465 to 1.252)	0.559
ribosomal protein L4	0.909 (0.632 to 1.437)	0.632
ribosomal protein L5	1.017 (0.693 to 1.491)	0.972
ribosomal protein L6	0.776 (0.4 to 1.161)	0.528

ribosomal protein L7	1.065 (0.928 to 1.222)	0.728
ribosomal protein L7a	1.019 (0.701 to 1.483)	0.968
ribosomal protein L8	0.778 (0.628 to 0.904)	0.0592
ribosomal protein L9	1	no replicates
ribosomal protein S10	0.978 (0.829 to 1.179)	0.85
ribosomal protein S11	0.939 (0.612 to 1.292)	0.725
ribosomal protein S12	1.327 (0.664 to 3.28)	0.338
ribosomal protein S13	1.016 (0.881 to 1.267)	0.823
ribosomal protein S14	1.066 (0.921 to 1.327)	0.476
ribosomal protein S15	1.051 (0.757 to 1.435)	0.728
ribosomal protein S15a	1.097 (0.582 to 2.067)	0.908
ribosomal protein S17	1.018 (0.529 to 1.455)	0.922
ribosomal protein S19	1.459 (1.002 to 2.882)	0.384
ribosomal protein S2	0.802 (0.511 to 1.257)	0.709
ribosomal protein S20	0.997 (0.711 to 1.291)	0.985
ribosomal protein S23	0.83 (0.755 to 0.914)	0.302
ribosomal protein S24	0.984 (0.679 to 1.392)	0.908
ribosomal protein S25	1.132 (0.883 to 1.436)	0.244
ribosomal protein S26	1.124 (1.042 to 1.214)	0.367
ribosomal protein S27 (metallopanstimulin 1)	1.034 (0.799 to 1.338)	0.918
ribosomal protein S27a	0.936 (0.768 to 1.141)	0.794
ribosomal protein S28	1.639 (1.251 to 3.748)	0.0763
ribosomal protein S29	1.311 (1.28 to 1.367)	0.00579
ribosomal protein S3	1.394 (0.867 to 1.928)	0.304
ribosomal protein S4, X-linked	1.37 (0.94 to 4.605)	0.362
ribosomal protein S4, Y-linked	1.521 (1.051 to 2.076)	0.169
ribosomal protein S6	0.951 (0.518 to 1.846)	0.836
ribosomal protein S6 kinase, 70kDa, polypeptide 1	0.823 (0.787 to 0.86)	0.143
ribosomal protein S6 kinase, 70kDa, polypeptide 2	1.136 (0.971 to 1.332)	0.296
ribosomal protein S6 kinase, 90kDa, polypeptide 3	1.093 (1.048 to 1.139)	0.28
ribosomal protein S7	1.056 (0.806 to 1.172)	0.472
ribosomal protein S8	0.727 (0.212 to 1.53)	0.659
ribosomal protein, large P2	1.212 (0.904 to 1.971)	0.257
ribosomal protein, large, P0	0.841 (0.776 to 0.943)	0.0251
ribosomal protein, large, P1	0.967 (0.74 to 1.282)	0.751
ribosome binding protein 1 homolog 180kDa (dog)	0.711 (0.387 to 1.577)	0.393
ring finger protein (C3H2C3 type) 6	1.72 (1.308 to 2.261)	0.297
ring finger protein 1	1.444 (0.484 to 3.664)	0.318
ring finger protein 14	1.03 (0.867 to 1.582)	0.804
ring finger protein 2	1.455 (1.11 to 1.906)	0.398
ring finger protein 4	0.414 (0.303 to 0.566)	0.216
ring finger protein 5	1.741 (0.756 to 4.271)	0.384
RIO kinase 3 (yeast)	1.236 (1.029 to 1.484)	0.453
RNA (guanine-7-) methyltransferase	1.115 (0.152 to 2.918)	0.882
RNA binding motif protein 3	1.229 (0.774 to 1.885)	0.508
RNA binding motif protein 4	1.374 (1 to 1.654)	0.185
RNA binding motif protein, X chromosome	1.075 (0.603 to 2.586)	0.885
RNA binding motif protein, Y chromosome, family 1, member A1	1.413 (0.896 to 1.936)	0.276
RNA binding motif protein, Y chromosome, family 2, member B	0.931 (0.66 to 1.311)	0.868
RNA binding motif, single stranded interacting protein 1	1.234 (1.032 to 1.525)	0.206
RNA binding motif, single stranded interacting protein 2	1.39 (1.187 to 1.628)	0.285
RNA guanylyltransferase and 5'-phosphatase	1.102 (0.713 to 1.702)	0.86
RNA helicase	0.775 (0.595 to 1.01)	0.513
RTC domain containing 1	1.362 (1.073 to 1.729)	0.419
runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)	0.877 (0.77 to 1)	0.5
runt-related transcription factor 3	0.662 (0.601 to 0.73)	0.148
RuvB-like 1 (E. coli)	1.078 (1 to 1.17)	0.238
ryanodine receptor 2 (cardiac)	1.01 (0.984 to 1.036)	0.767
ryanodine receptor 3	0.92 (0.734 to 1.153)	0.774
S100 calcium binding protein A10 (annexin II ligand, calpactin I, light polypeptide (p11))	0.987 (0.613 to 1.804)	0.971
S100 calcium binding protein A11 (calgizzarin)	0.804 (0.686 to 0.943)	0.402
S100 calcium binding protein A12 (calgranulin C)	1.789 (0.941 to 3.402)	0.532
S100 calcium binding protein A13	1.479 (1 to 2.125)	0.214
S100 calcium binding protein A2	0.782 (0.431 to 1.277)	0.52
S100 calcium binding protein A3	0.731 (0.502 to 1.066)	0.559
S100 calcium binding protein A4 (calcium protein, calvasculin, metastasin, murine placental homolog)	1.002 (0.673 to 1.493)	0.997
S100 calcium binding protein A5	1.064 (0.957 to 1.2)	0.444

S100 calcium binding protein P	1	no replicates
S100 calcium binding protein, beta (neural)	1.045 (0.729 to 1.308)	0.753
SA hypertension-associated homolog (rat)	0.789 (0.601 to 1.268)	0.425
S-adenosylhomocysteine hydrolase-like 1	0.867 (0.594 to 1.266)	0.771
S-antigen; retina and pineal gland (arrestin)	1.054 (0.491 to 2.099)	0.835
sarcoglycan, alpha (50kDa dystrophin-associated glycoprotein)	1.036 (0.805 to 1.333)	0.912
sarcoglycan, beta (43kDa dystrophin-associated glycoprotein)	1.049 (1.026 to 1.071)	0.269
sarcoglycan, delta (35kDa dystrophin-associated glycoprotein)	1.037 (1.031 to 1.043)	0.0999
sarcoglycan, gamma (35kDa dystrophin-associated glycoprotein)	0.958 (0.926 to 0.99)	0.421
sarcolipin	1.062 (0.767 to 1.378)	0.761
sarcosine dehydrogenase	0.954 (0.839 to 1.086)	0.778
scaffold attachment factor B	0.833 (0.698 to 1.043)	0.263
scavenger receptor class B, member 1	0.91 (0.826 to 1.002)	0.508
scavenger receptor class B, member 2	0.898 (0.804 to 1)	0.23
scavenger receptor class F, member 1	0.788 (0.759 to 0.819)	0.101
sciellin	1.127 (1.123 to 1.131)	0.0167
SCO cytochrome oxidase deficient homolog 1 (yeast)	1.059 (1.012 to 1.108)	0.425
SEC14-like 1 (S. cerevisiae)	0.67 (0.417 to 1)	0.256
secreted and transmembrane 1	1.201 (0.989 to 1.396)	0.213
secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	1.194 (1.144 to 1.247)	0.151
secreted phosphoprotein 2, 24kDa	1.105 (0.953 to 1.282)	0.621
secretin receptor	0.909 (0.476 to 1.558)	0.654
secretoglobin, family 1A, member 1 (uteroglobin)	1.576 (1.188 to 2.092)	0.354
secretoglobin, family 2A, member 1	1.554 (1.3 to 1.858)	0.245
secretoglobin, family 2A, member 2	0.993 (0.988 to 0.997)	0.361
secretory leukocyte protease inhibitor (antileukoproteinase)	1.326 (1.103 to 1.76)	0.188
sel-1 suppressor of lin-12-like (C. elegans)	1.165 (0.807 to 1.52)	0.266
selectin L (lymphocyte adhesion molecule 1)	0.942 (0.765 to 1.162)	0.429
selectin P (granule membrane protein 140kDa, antigen CD62)	2.002 (1.458 to 2.747)	0.273
selectin P ligand	1.05 (0.927 to 1.19)	0.762
selenoprotein P, plasma, 1	1.009 (0.91 to 1.12)	0.944
selenoprotein W, 1	0.805 (0.461 to 1.407)	0.764
sema domain, immunoglobulin domain (Ig), and GPI membrane anchor, (semaphorin) 7A	1.426 (1.267 to 1.605)	0.205
sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B	1.636 (1.028 to 3.623)	0.343
semenogelin II	0.884 (0.842 to 0.962)	0.101
sepiapterin reductase (7,8-dihydrobiopterin:NADP+ oxidoreductase)	0.919 (0.915 to 0.923)	0.0318
serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 2	1.551 (0.803 to 4.098)	0.47
serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4	0.908 (0.79 to 1.044)	0.615
serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6	0.833 (0.409 to 1.549)	0.578
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 10	1.214 (1.112 to 1.325)	0.271
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	0.53	no replicates
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 7	1.21 (1.091 to 1.276)	0.0663
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 9	0.958 (0.789 to 1.196)	0.759
serine (or cysteine) proteinase inhibitor, clade C (antithrombin), member 1	1.083 (0.777 to 1.509)	0.851
serine (or cysteine) proteinase inhibitor, clade D (heparin cofactor), member 1	0.882 (0.736 to 1.058)	0.615
serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	1.101 (0.811 to 1.444)	0.624
serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)	1.098 (1.073 to 1.123)	0.153
serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	0.969 (0.566 to 1.458)	0.922
serine hydroxymethyltransferase 2 (mitochondrial)	1.093 (0.609 to 1.962)	0.904
serine protease inhibitor, Kazal type 1	1.166 (1.029 to 1.321)	0.435
serine protease inhibitor, Kunitz type 1	1.077 (1.021 to 1.136)	0.397
serine/threonine kinase 10	0.852 (0.721 to 1.006)	0.512
serine/threonine kinase 11 (Peutz-Jeghers syndrome)	0.975 (0.78 to 1.254)	0.872
serine/threonine kinase 13 (aurora/IPL1-like)	1.378 (1.134 to 1.676)	0.348
serine/threonine kinase 16	1.076 (1.014 to 1.141)	0.433
serine/threonine kinase 24 (STE20 homolog, yeast)	1.316 (1.108 to 1.606)	0.126
serine/threonine kinase 4	0.51 (0.255 to 1.019)	0.509
serine/threonine kinase 6	1.237 (0.671 to 2.553)	0.495
serum amyloid A1	0.957 (0.586 to 1.444)	0.883
serum deprivation response (phosphatidylserine binding protein)	0.871 (0.223 to 2.086)	0.8

serum/glucocorticoid regulated kinase	0.799 (0.685 to 0.932)	0.383
seryl-tRNA synthetase	0.818 (0.309 to 1.682)	0.73
SET domain and mariner transposase fusion gene	1.488 (1.033 to 2.806)	0.174
SET translocation (myeloid leukemia-associated)	1.026 (0.797 to 1.43)	0.896
seven in absentia homolog 2 (Drosophila)	1.185 (0.644 to 2.181)	0.827
sex comb on midleg-like 1 (Drosophila)	0.843 (0.564 to 1.26)	0.744
sex determining region Y	0.84 (0.832 to 0.848)	0.0352
sex hormone-binding globulin	0.858 (0.57 to 1.075)	0.534
SFRS protein kinase 2	1.167 (0.975 to 1.395)	0.547
SH2 domain protein 1A, Duncan's disease (lymphoproliferative syndrome)	1.028 (0.884 to 1.195)	0.884
SH3 domain binding glutamic acid-rich protein	1.403 (1.183 to 1.664)	0.297
SH3 domain binding glutamic acid-rich protein like	1.019 (0.927 to 1.119)	0.877
SH3-domain GRB2-like 1	1.014 (0.791 to 1.301)	0.964
SH3-domain GRB2-like 2	0.491 (0.448 to 0.537)	0.0808
SH3-domain GRB2-like 3	1.052 (0.812 to 1.639)	0.842
short stature homeobox	1.228 (1.091 to 1.367)	0.0215
short stature homeobox 2	0.621 (0.46 to 0.97)	0.171
sialic acid binding Ig-like lectin 6	1.892 (1.242 to 2.54)	0.0982
sialidase 1 (lysosomal sialidase)	1.107 (0.76 to 1.55)	0.592
sialidase 2 (cytosolic sialidase)	1.313 (0.946 to 1.822)	0.559
sialyltransferase 1 (beta-galactoside alpha-2,6-sialyltransferase)	1.547 (1.182 to 1.874)	0.0207
sialyltransferase 4B (beta-galactoside alpha-2,3-sialyltransferase)	1.377	no replicates
sialyltransferase 4C (beta-galactoside alpha-2,3-sialyltransferase)	2.365 (1.051 to 8.507)	0.315
sialyltransferase 6 (N-acetyllacosaminide alpha 2,3-sialyltransferase)	1.344 (1.107 to 1.632)	0.369
sialyltransferase 8A (alpha-N-acetylneuraminate: alpha-2,8-sialyltransferase, GD3 synthase)	1.067 (1.054 to 1.08)	0.119
signal recognition particle 14kDa (homologous Alu RNA binding protein)	1.443 (1.142 to 1.823)	0.361
signal recognition particle 19kDa	1.15 (0.642 to 1.627)	0.538
signal recognition particle 54kDa	0.982 (0.647 to 1.441)	0.944
signal recognition particle 72kDa	1.148 (0.834 to 1.376)	0.19
signal recognition particle 9kDa	0.895 (0.826 to 0.97)	0.398
signal recognition particle receptor ('docking protein')	1.682 (1.051 to 2.692)	0.468
signal sequence receptor, alpha (translocon-associated protein alpha)	1.884 (0.87 to 4.08)	0.563
signal sequence receptor, beta (translocon-associated protein beta)	0.922 (0.883 to 0.963)	0.311
signal sequence receptor, gamma (translocon-associated protein gamma)	0.913 (0.845 to 0.987)	0.449
signal transducer and activator of transcription 1, 91kDa	0.546	no replicates
signal transducer and activator of transcription 4	1.028 (0.541 to 1.738)	0.917
signal transducer and activator of transcription 6, interleukin-4 induced	1.019 (0.685 to 1.38)	0.876
signal transducing adaptor molecule (SH3 domain and ITAM motif) 1	0.839 (0.454 to 1.281)	0.632
signal-induced proliferation-associated gene 1	1.029 (0.576 to 1.292)	0.892
signaling lymphocytic activation molecule	0.952 (0.585 to 1.687)	0.799
silver homolog (mouse)	1.011 (0.86 to 1.338)	0.947
sin3-associated polypeptide, 30kDa	0.878 (0.858 to 0.898)	0.112
sine oculis homeobox homolog 1 (Drosophila)	1.187 (0.867 to 1.625)	0.683
sine oculis homeobox homolog 3 (Drosophila)	1.604	no replicates
sine oculis homeobox homolog 6 (Drosophila)	0.943 (0.559 to 1.588)	0.928
single-minded homolog 1 (Drosophila)	0.844 (0.786 to 0.907)	0.255
single-stranded DNA binding protein	1.066 (0.988 to 1.15)	0.556
Sjogren syndrome antigen A1 (52kDa, ribonucleoprotein autoantigen SS-A/Ro)	0.864 (0.858 to 0.869)	0.0298
Sjogren syndrome antigen A2 (60kDa, ribonucleoprotein autoantigen SS-A/Ro)	0.831 (0.632 to 1.385)	0.544
Sjogren's syndrome nuclear autoantigen 1	0.909 (0.69 to 1.414)	0.711
SKI-like	1.673 (1.289 to 2.226)	0.0829
SLC9A2=Na+/H+ exchanger	1.197 (0.756 to 2.117)	0.613
slit homolog 1 (Drosophila)	1.066 (0.9 to 1.295)	0.374
small glutamine-rich tetratricopeptide repeat (TPR)-containing	1	no replicates
small nuclear ribonucleoprotein 70kDa polypeptide (RNP antigen)	0.968 (0.637 to 1.679)	0.919
small nuclear ribonucleoprotein D1 polypeptide 16kDa	1.072 (0.998 to 1.202)	0.356
small nuclear ribonucleoprotein D2 polypeptide 16.5kDa	0.947 (0.783 to 1.145)	0.823
small nuclear ribonucleoprotein D3 polypeptide 18kDa	0.936 (0.866 to 1.012)	0.551
small nuclear ribonucleoprotein polypeptide A	1.244 (1.104 to 1.401)	0.318
small nuclear ribonucleoprotein polypeptide A'	0.871 (0.841 to 0.902)	0.158
small nuclear ribonucleoprotein polypeptide B''	1.081 (1.073 to 1.088)	0.0556
small nuclear ribonucleoprotein polypeptide C	1.167 (1.15 to 1.185)	0.0598
small nuclear ribonucleoprotein polypeptide E	1.322 (1.171 to 1.492)	0.261
small nuclear ribonucleoprotein polypeptide F	0.982 (0.899 to 1.073)	0.872
small nuclear ribonucleoprotein polypeptide G	0.747 (0.209 to 1.639)	0.694

small nuclear ribonucleoprotein polypeptide N	1.207 (1 to 1.522)	0.267
small nuclear ribonucleoprotein polypeptides B and B1	0.865 (0.587 to 1.183)	0.553
small nuclear RNA activating complex, polypeptide 1, 43kDa	0.86 (0.824 to 0.899)	0.18
small nuclear RNA activating complex, polypeptide 2, 45kDa	1.273 (1.199 to 1.352)	0.156
small nuclear RNA activating complex, polypeptide 3, 50kDa	1.026 (0.933 to 1.129)	0.831
small nuclear RNA activating complex, polypeptide 4, 190kDa	1.021 (0.619 to 1.683)	0.974
small proline-rich protein 1B (cornifin)	0.959 (0.831 to 1.107)	0.819
small proline-rich protein 2B	0.843 (0.679 to 1.046)	0.574
small proline-rich protein 3	2.095 (2.001 to 2.195)	0.0398
Smcy homolog, Y chromosome (mouse)	1.927 (1.06 to 4.154)	0.246
smoothelin	1.628 (0.701 to 3.767)	0.269
SMT3 suppressor of mif two 3 homolog 1 (yeast)	0.994 (0.874 to 1.132)	0.972
SMT3 suppressor of mif two 3 homolog 2 (yeast)	0.979 (0.849 to 1.128)	0.905
snail homolog 1 (Drosophila)	0.953 (0.852 to 1.066)	0.74
sodium channel, nonvoltage-gated 1 alpha	1.042 (0.962 to 1.129)	0.695
sodium channel, nonvoltage-gated 1, delta	0.728 (0.485 to 1.021)	0.282
sodium channel, nonvoltage-gated 1, gamma	1.17 (0.956 to 1.317)	0.261
sodium channel, voltage-gated, type I, beta	0.968 (0.332 to 2.382)	0.93
sodium channel, voltage-gated, type II, beta	1.04 (0.844 to 1.456)	0.84
sodium channel, voltage-gated, type IX, alpha	1.04 (0.812 to 1.333)	0.899
sodium channel, voltage-gated, type VII, alpha	1.566 (1.294 to 1.894)	0.256
sodium channel, voltage-gated, type X, alpha	1.347 (0.601 to 2.587)	0.293
solute carrier family 1 (glial high affinity glutamate transporter), member 2	1.278 (1.077 to 1.517)	0.388
solute carrier family 1 (glial high affinity glutamate transporter), member 3	1.041 (0.636 to 1.403)	0.831
solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6	1.339 (1.218 to 1.472)	0.2
solute carrier family 1 (neutral amino acid transporter), member 5	1.52 (1.106 to 2.726)	0.288
solute carrier family 10 (sodium/bile acid cotransporter family), member 1	1.144 (1 to 1.518)	0.254
solute carrier family 10 (sodium/bile acid cotransporter family), member 2	1.236 (0.916 to 1.681)	0.35
solute carrier family 12 (potassium/chloride transporters), member 4	1.232 (0.94 to 1.614)	0.581
solute carrier family 12 (sodium/chloride transporters), member 3	1.096 (0.815 to 1.475)	0.808
solute carrier family 12 (sodium/potassium/chloride transporters), member 1	0.609	no replicates
solute carrier family 12 (sodium/potassium/chloride transporters), member 2	1.044 (0.728 to 1.497)	0.925
solute carrier family 16 (monocarboxylic acid transporters), member 2 (putative transporter)	1.083 (0.858 to 1.738)	0.654
solute carrier family 17 (sodium phosphate), member 1	1.097 (0.819 to 1.498)	0.647
solute carrier family 18 (vesicular monoamine), member 1	0.912 (0.817 to 1.017)	0.554
solute carrier family 19 (folate transporter), member 1	1.141 (1.027 to 1.268)	0.43
solute carrier family 2 (facilitated glucose transporter), member 4	0.855 (0.82 to 0.892)	0.169
solute carrier family 2 (facilitated glucose/fructose transporter), member 5	1.016 (0.79 to 1.307)	0.959
solute carrier family 20 (phosphate transporter), member 1	0.96 (0.548 to 1.395)	0.816
solute carrier family 20 (phosphate transporter), member 2	1.866 (1.11 to 3.136)	0.442
solute carrier family 21 (organic anion transporter), member 3	1.062 (0.854 to 1.321)	0.829
solute carrier family 21 (prostaglandin transporter), member 2	0.962 (0.859 to 1.078)	0.793
solute carrier family 22 (organic cation transporter), member 1-like	0.977 (0.854 to 1.2)	0.844
solute carrier family 22 (organic cation transporter), member 1-like antisense	1.304 (1 to 1.953)	0.171
solute carrier family 22 (organic cation transporter), member 2	1.413 (0.932 to 2.141)	0.558
solute carrier family 22 (organic cation transporter), member 4	0.965 (0.895 to 1.041)	0.719
solute carrier family 22 (organic cation transporter), member 5	1.092 (0.847 to 1.373)	0.449
solute carrier family 25 (carnitine/acylcarnitine translocase), member 20	0.554 (0.155 to 1.265)	0.457
solute carrier family 25 (mitochondrial carrier, Aralar), member 12	1.221 (1 to 1.491)	0.501
solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5	1.271 (1.091 to 1.482)	0.362
solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11	1.298 (1.04 to 1.852)	0.284
solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3	0.899 (0.648 to 1.439)	0.478
solute carrier family 26, member 3	0.935 (0.654 to 1.249)	0.756
solute carrier family 26, member 4	1.007 (0.734 to 1.75)	0.983
solute carrier family 27 (fatty acid transporter), member 2	0.858 (0.727 to 1.012)	0.523
solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	1.098 (1.096 to 1.099)	0.00972
solute carrier family 3 (cystine, dibasic and neutral amino acid transporters, activator of cystine, dibasic and neutral amino acid transport), member 1	0.595 (0.158 to 1.244)	0.516
solute carrier family 30 (zinc transporter), member 3	1.733 (0.951 to 9.809)	0.412
solute carrier family 31 (copper transporters), member 1	0.524 (0.191 to 1.042)	0.336
solute carrier family 31 (copper transporters), member 2	0.861 (0.705 to 0.999)	0.285

solute carrier family 33 (acetyl-CoA transporter), member 1	2.98	no replicates
solute carrier family 34 (sodium phosphate), member 1	1.1 (0.855 to 1.416)	0.77
solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3, Diego blood group)	0.653 (0.52 to 0.819)	0.312
solute carrier family 4, anion exchanger, member 2 (erythrocyte membrane protein band 3-like 1)	1.359 (1.088 to 1.697)	0.399
solute carrier family 4, sodium bicarbonate cotransporter, member 4	1.091 (0.61 to 2.452)	0.855
solute carrier family 5 (inositol transporters), member 3	0.927 (0.864 to 0.994)	0.475
solute carrier family 5 (sodium iodide symporter), member 5	0.659 (0.247 to 1.047)	0.302
solute carrier family 5 (sodium/glucose cotransporter), member 1	1.072 (0.772 to 1.274)	0.504
solute carrier family 6 (neurotransmitter transporter), member 14	0.963 (0.765 to 1.212)	0.896
solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12	1.143 (1.032 to 1.266)	0.416
solute carrier family 6 (neurotransmitter transporter, creatine), member 8	0.712 (0.693 to 0.732)	0.0511
solute carrier family 6 (neurotransmitter transporter, GABA), member 1	0.947 (0.902 to 0.995)	0.47
solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2	0.821 (0.548 to 1.012)	0.435
solute carrier family 7 (cationic amino acid transporter, y+ system), member 1	0.771 (0.62 to 0.958)	0.442
solute carrier family 7 (cationic amino acid transporter, y+ system), member 2	1.259 (1 to 1.483)	0.191
solute carrier family 9 (sodium/hydrogen exchanger), isoform 5	0.872 (0.793 to 1.004)	0.197
somatostatin	1.053 (0.621 to 1.797)	0.828
somatostatin receptor 1	1.811 (1.051 to 3.121)	0.472
somatostatin receptor 3	1.321 (1.085 to 1.608)	0.392
somatostatin receptor 4	0.919 (0.635 to 1.33)	0.857
somatostatin receptor 5	0.993 (0.56 to 1.898)	0.986
SON DNA binding protein	1.397 (1.155 to 1.691)	0.33
son of sevenless homolog 2 (Drosophila)	0.752 (0.372 to 1.165)	0.507
sorcin	1.316 (1.218 to 1.601)	0.0248
sortilin-related receptor, L(DLR class) A repeats-containing	0.91 (0.654 to 1.267)	0.823
sorting nexin 1	0.668 (0.616 to 0.725)	0.127
sorting nexin 2	1.19 (1.137 to 1.246)	0.164
sorting nexin 3	0.998 (0.928 to 1.072)	0.98
sorting nexin 4	1.262 (0.946 to 2.157)	0.477
SP110 nuclear body protein	0.94 (0.36 to 1.697)	0.73
Sp2 transcription factor	0.748 (0.484 to 1.166)	0.371
Sp4 transcription factor	0.682 (0.59 to 0.788)	0.23
sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican)	0.813 (0.734 to 0.899)	0.289
spastic paraplegia 7, paraplegin (pure and complicated autosomal recessive)	1.121 (0.969 to 1.298)	0.578
speckle-type POZ protein	0.844 (0.543 to 1.094)	0.354
spectrin, alpha, erythrocytic 1 (elliptocytosis 2)	1.29 (1.284 to 1.296)	0.0108
spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	1.163 (1.068 to 1.265)	0.326
spectrin, beta, erythrocytic (includes spherocytosis, clinical type I)	0.999 (0.919 to 1.075)	0.985
spectrin, beta, non-erythrocytic 1	0.929 (0.853 to 1.012)	0.548
spectrin, beta, non-erythrocytic 2	0.96 (0.913 to 1.01)	0.57
sperm specific antigen 2	1.257 (0.962 to 1.643)	0.55
spermidine synthase	1.107 (0.957 to 1.28)	0.613
spermidine/spermine N1-acetyltransferase	0.646 (0.554 to 0.754)	0.217
spermine synthase	1.267 (1 to 1.599)	0.121
S-phase kinase-associated protein 1A (p19A)	1.154 (0.356 to 3.369)	0.655
S-phase kinase-associated protein 2 (p45)	1.425 (1 to 2.235)	0.125
sphingomyelin phosphodiesterase 1, acid lysosomal (acid sphingomyelinase)	0.857 (0.646 to 1.099)	0.288
sphingomyelin phosphodiesterase 2, neutral membrane (neutral sphingomyelinase)	2.138 (1.514 to 3.019)	0.271
Spi-B transcription factor (Spi-1/PU.1 related)	1.049 (0.942 to 1.169)	0.734
spinocerebellar ataxia 1 (olivopontocerebellar ataxia 1, autosomal dominant, ataxin 1)	1.448 (0.902 to 2.955)	0.171
spinocerebellar ataxia 2 (olivopontocerebellar ataxia 2, autosomal dominant, ataxin 2)	1.015 (0.999 to 1.046)	0.431
spinocerebellar ataxia 7 (olivopontocerebellar atrophy with retinal degeneration)	0.884 (0.608 to 1.285)	0.797
spleen tyrosine kinase	1.162 (0.981 to 1.452)	0.326
splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	0.92 (0.846 to 1.002)	0.507
splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	0.861 (0.748 to 0.991)	0.48
splicing factor, arginine/serine-rich 2	1.385 (0.753 to 2.548)	0.688
splicing factor, arginine/serine-rich 3	1.08 (1.024 to 1.138)	0.383

splicing factor, arginine/serine-rich 4	1.556 (1.271 to 2.298)	0.151
splicing factor, arginine/serine-rich 5	0.748 (0.525 to 0.926)	0.245
splicing factor, arginine/serine-rich 7, 35kDa	0.92 (0.795 to 1.063)	0.667
splicing factor, arginine/serine-rich 8 (suppressor-of-white-apricot homolog, Drosophila)	1.293 (0.609 to 2.58)	0.506
splicing factor, arginine/serine-rich 9	1.121 (0.904 to 1.588)	0.42
squalene epoxidase	0.912 (0.871 to 0.954)	0.289
src family associated phosphoprotein 1	1.003 (0.804 to 1.387)	0.983
Src-like-adaptor	0.8 (0.535 to 1.59)	0.584
SRY (sex determining region Y)-box 1	1.033 (0.919 to 1.161)	0.829
SRY (sex determining region Y)-box 10	1.176 (0.805 to 2.433)	0.451
SRY (sex determining region Y)-box 11	1.432 (1.164 to 1.774)	0.0985
SRY (sex determining region Y)-box 12	1.083 (0.685 to 1.406)	0.649
SRY (sex determining region Y)-box 14	0.732 (0.684 to 0.779)	0.0142
SRY (sex determining region Y)-box 15	1.183 (0.95 to 1.563)	0.371
SRY (sex determining region Y)-box 3	0.939 (0.429 to 2.761)	0.856
SRY (sex determining region Y)-box 4	0.689 (0.638 to 0.743)	0.128
SRY (sex determining region Y)-box 5	1.318 (0.675 to 2.845)	0.42
SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	1.008 (0.672 to 1.399)	0.964
stannin	1.315 (1.181 to 1.464)	0.239
stanniocalcin 1	0.804 (0.305 to 1.748)	0.712
stanniocalcin 2	1 (0.794 to 1.148)	0.999
statherin	1.219 (1.156 to 1.286)	0.167
stathmin 1/oncoprotein 18	0.926 (0.53 to 1.408)	0.755
staufen, RNA binding protein (Drosophila)	1.066 (0.663 to 1.645)	0.753
steroid sulfatase (microsomal), arylsulfatase C, isozyme S	1.035 (0.898 to 1.201)	0.61
steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1)	1.267 (0.828 to 1.939)	0.677
steroid-5-alpha-reductase, alpha polypeptide 2 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 2)	0.627 (0.232 to 1.198)	0.452
steroidogenic acute regulatory protein	0.749 (0.506 to 1.108)	0.595
sterol carrier protein 2	0.882 (0.511 to 1.266)	0.695
sterol regulatory element binding transcription factor 1	4.529 (4.164 to 4.926)	0.0354
sterol regulatory element binding transcription factor 2	1.154 (1.065 to 1.276)	0.116
sterol-C4-methyl oxidase-like	0.866 (0.816 to 0.919)	0.248
stomatin	1.415 (0.927 to 2.161)	0.562
striatin, calmodulin binding protein	0.815	no replicates
stromal cell-derived factor 2	0.775 (0.556 to 1.036)	0.1
stromal interaction molecule 1	1.016 (0.896 to 1.153)	0.919
structure specific recognition protein 1	0.901 (0.688 to 1.096)	0.534
succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	1.644 (1.241 to 2.25)	0.102
succinate dehydrogenase complex, subunit C, integral membrane protein, 15kDa	1.171 (0.76 to 1.984)	0.631
succinate dehydrogenase complex, subunit D, integral membrane protein	1.049 (0.942 to 1.169)	0.733
sucrase-isomaltase	1.918 (1.256 to 2.929)	0.367
sulfite oxidase	1.029 (0.702 to 1.258)	0.793
sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	0.979 (0.818 to 1.173)	0.926
sulfotransferase family, cytosolic, 1A, phenol-preferring, member 2	1.033 (0.972 to 1.098)	0.687
sulfotransferase family, cytosolic, 1C, member 1	0.668 (0.475 to 0.94)	0.447
sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA) - preferring, member 1	1.032 (0.874 to 1.218)	0.775
sulfotransferase family, cytosolic, 2B, member 1	1.07 (0.941 to 1.217)	0.69
sulfotransferase, estrogen-preferring	1.22 (1.128 to 1.319)	0.239
superkiller viralicidic activity 2-like (S. cerevisiae)	1.639 (1.244 to 2.352)	0.0401
superoxide dismutase 3, extracellular	1.096 (0.955 to 1.258)	0.627
supervillin	1.636 (1.057 to 2.531)	0.462
suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein)	1.058 (1.006 to 1.113)	0.466
suppression of tumorigenicity 5	1.126 (0.83 to 1.527)	0.764
suppressor of cytokine signaling 1	0.794 (0.636 to 1.217)	0.393
suppressor of Ty 3 homolog (S. cerevisiae)	1.329 (1.021 to 1.532)	0.164
suppressor of Ty 4 homolog 1 (S. cerevisiae)	1.022 (0.872 to 1.389)	0.899
suppressor of Ty 5 homolog (S. cerevisiae)	1.096 (0.803 to 1.94)	0.779
suppressor of variegation 3-9 homolog 1 (Drosophila)	1.264 (1.201 to 1.33)	0.137
surfactant, pulmonary-associated protein A2	1.17 (0.81 to 2.432)	0.573
surfactant, pulmonary-associated protein B	1.578 (0.896 to 2.819)	0.302
surfactant, pulmonary-associated protein C	1.056 (0.797 to 1.26)	0.741
surfactant, pulmonary-associated protein D	1.052 (0.58 to 1.589)	0.883
surfeit 1	0.919 (0.872 to 0.969)	0.355
surfeit 5	1.01 (0.969 to 1.053)	0.851

survival of motor neuron 1, telomeric	1.062 (0.767 to 1.359)	0.653
survival of motor neuron protein interacting protein 1	0.692 (0.471 to 1.016)	0.514
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	1.044 (0.713 to 1.25)	0.695
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 3	0.973 (0.819 to 1.156)	0.9
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	1.562 (0.701 to 2.574)	0.385
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 1	0.676 (0.415 to 1.109)	0.301
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2	0.922 (0.902 to 0.942)	0.168
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1	0.98 (0.959 to 1.001)	0.508
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2	0.994 (0.845 to 1.17)	0.978
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	1.357 (1.01 to 1.824)	0.49
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1	1.041 (0.876 to 1.221)	0.511
synapsin I	1.003 (1 to 1.007)	0.465
synapsin II	1.017 (0.744 to 1.944)	0.943
synapsin III	3.467 (2.459 to 4.889)	0.172
synaptobrevin-like 1	1.414 (1 to 2.103)	0.25
synaptonemal complex protein 1	1.216 (0.891 to 1.659)	0.643
synaptophysin-like protein	0.738 (0.409 to 1.144)	0.427
synaptosomal-associated protein, 23kDa	1.719	no replicates
synaptotagmin I	1.294 (1.061 to 1.58)	0.418
synaptotagmin V	1.02 (0.911 to 1.16)	0.804
syndecan 1	1.162 (1 to 1.501)	0.218
syndecan 4 (amphiglycan, ryudocan)	1.125 (0.737 to 1.718)	0.828
syndecan binding protein (syntenin)	0.933 (0.71 to 1.36)	0.678
synovial sarcoma translocation, chromosome 18	0.914 (0.726 to 1.054)	0.518
synovial sarcoma, X breakpoint 1	1.153 (0.888 to 1.498)	0.683
synovial sarcoma, X breakpoint 4	1.199 (0.713 to 2.015)	0.786
syntaxin 10	0.764 (0.7 to 0.834)	0.2
syntaxin 11	0.755 (0.668 to 0.855)	0.264
syntaxin 16	1.09 (0.743 to 1.599)	0.859
syntaxin 1A (brain)	1.529 (1.024 to 2.673)	0.279
syntaxin 1B1	1.274 (1 to 1.48)	0.0805
syntaxin 3A	1.81 (1.514 to 2.238)	0.00578
syntaxin 4A (placental)	1.143 (0.502 to 1.786)	0.776
syntaxin 5A	1.129 (0.99 to 1.39)	0.367
syntaxin 7	1.231 (0.522 to 2.904)	0.849
syntaxin binding protein 1	0.809 (0.641 to 1.153)	0.36
syntaxin binding protein 2	0.892 (0.565 to 1.147)	0.668
syntaxin binding protein 3	0.697 (0.542 to 1.028)	0.208
syntrophin, alpha 1 (dystrophin-associated protein A1, 59kDa, acidic component)	1.459 (1.205 to 1.766)	0.298
syntrophin, beta 2 (dystrophin-associated protein A1, 59kDa, basic component 2)	0.997 (0.774 to 1.391)	0.98
synuclein, alpha (non A4 component of amyloid precursor)	1 (1 to 1)	no replicates
synuclein, beta	1.08 (0.833 to 1.4)	0.817
synuclein, gamma (breast cancer-specific protein 1)	1.226 (0.959 to 1.567)	0.559
T, brachyury homolog (mouse)	1.181 (1.08 to 1.291)	0.314
tachykinin, precursor 1 (substance K, substance P, neurokinin 1, neurokinin 2, neuromedin L, neurokinin alpha, neuropeptide K, neuropeptide gamma)	0.776 (0.368 to 1.331)	0.577
TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 250kDa	1.032 (0.681 to 1.306)	0.894
TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 30kDa	0.98 (0.975 to 0.986)	0.174
TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 28kDa	0.726 (0.514 to 1)	0.238
TAF13 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 18kDa	1.089 (1.052 to 1.126)	0.242
TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68kDa	0.656 (0.554 to 0.755)	0.0432
TAF2 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 150kDa	1.078 (0.942 to 1.233)	0.677
TAF5 RNA polymerase II, TATA box binding protein (TBP)-associated	1.012 (0.817 to 1.254)	0.964

factor, 100kDa		
TAF6 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 80kDa	1.205 (0.943 to 1.526)	0.114
TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 55kDa	0.964 (0.896 to 1.106)	0.648
tafazzin (cardiomyopathy, dilated 3A (X-linked); endocardial fibroelastosis 2; Barth syndrome)	1.17 (0.635 to 2.177)	0.551
TAL1 (SCL) interrupting locus	1.496 (1.251 to 1.789)	0.266
talin 1	0.982 (0.885 to 1.09)	0.891
TAR (HIV) RNA binding protein 1	0.703 (0.231 to 1.251)	0.591
TAR (HIV) RNA binding protein 2	1.15 (0.819 to 1.701)	0.579
TATA box binding protein	0.947 (0.801 to 1.12)	0.801
T-box 1	1.028 (0.762 to 1.39)	0.888
T-box 10	1.645 (1.528 to 1.77)	0.0935
T-box 2	1.203 (1.008 to 1.437)	0.487
T-box 5	1.04 (0.659 to 1.93)	0.854
T-box 6	0.73 (0.717 to 0.743)	0.037
T-cell acute lymphocytic leukemia 1	0.845 (0.804 to 0.889)	0.185
T-cell acute lymphocytic leukemia 2	1.416 (1.232 to 1.627)	0.242
T-cell leukemia, homeobox 2	0.95 (0.787 to 1.105)	0.592
t-complex 10 (mouse)	0.889 (0.756 to 1.2)	0.516
t-complex-associated-testis-expressed 1-like	1.074 (0.946 to 1.219)	0.675
t-complex-associated-testis-expressed 1-like 1	3.549 (2.811 to 4.48)	0.116
TEA domain family member 4	0.843 (0.762 to 0.933)	0.341
tectorin alpha	1.108 (1.091 to 1.125)	0.0957
telomerase-associated protein 1	0.533	no replicates
telomeric repeat binding factor 2	1.073 (0.914 to 1.352)	0.611
tenascin XB	1.249 (0.882 to 3.828)	0.472
testis enhanced gene transcript (BAX inhibitor 1)	0.976 (0.903 to 1.055)	0.808
testis specific protein 1 (probe H4-1 p3-1)	0.96 (0.722 to 1.587)	0.836
testis-specific kinase 1	1.082	no replicates
tetranectin (plasminogen binding protein)	1.002 (0.392 to 2.504)	0.995
tetratricopeptide repeat domain 1	0.903 (0.715 to 1.026)	0.473
tetratricopeptide repeat domain 4	1.193 (0.713 to 3.117)	0.634
TFIIA-alpha/beta-like factor	1.021 (0.989 to 1.054)	0.635
TGFB inducible early growth response	1.023 (0.882 to 1.186)	0.904
TGFB inducible early growth response 2	1.072 (1.056 to 1.088)	0.131
TGFB-induced factor (TALE family homeobox)	1.064 (0.798 to 1.442)	0.752
thimet oligopeptidase 1	1.561 (1.304 to 1.869)	0.244
thiopurine S-methyltransferase	1.21 (1.075 to 1.362)	0.353
threonyl-tRNA synthetase	1.1 (1.028 to 1.177)	0.395
thrombomodulin	1.261 (1.141 to 1.406)	0.0619
thrombospondin 3	0.891 (0.305 to 1.768)	0.851
thrombospondin 4	0.937 (0.795 to 1.103)	0.758
thromboxane A synthase 1 (platelet, cytochrome P450, family 5, subfamily A)	1.359 (1.239 to 1.49)	0.186
thromboxane A2 receptor	1.086 (0.898 to 1.435)	0.364
Thy-1 cell surface antigen	0.781 (0.297 to 1.476)	0.665
thymidine kinase 1, soluble	2.386 (1.126 to 8.978)	0.321
thymine-DNA glycosylase	1.345 (0.928 to 2.103)	0.102
thymopoietin	1.153 (0.918 to 1.449)	0.645
thyroglobulin	0.918 (0.915 to 0.921)	0.0253
thyroid hormone responsive (SPOT14 homolog, rat)	0.904 (0.89 to 0.919)	0.0985
thyroid peroxidase	0.768 (0.621 to 0.949)	0.431
thyroid stimulating hormone receptor	1.152 (0.995 to 1.335)	0.512
thyroid stimulating hormone, beta	1.248 (0.844 to 1.794)	0.416
thyroid transcription factor 1	0.891 (0.412 to 1.57)	0.799
thyrotrophic embryonic factor	1.095 (1.022 to 1.193)	0.0859
thyrotropin-releasing hormone	0.807 (0.619 to 1.051)	0.566
thyrotropin-releasing hormone receptor	0.916 (0.886 to 0.947)	0.232
TIA1 cytotoxic granule-associated RNA binding protein-like 1	1.507 (1.107 to 2.05)	0.41
tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	0.719 (0.444 to 0.981)	0.31
tissue specific transplantation antigen P35B	1.106 (0.801 to 1.689)	0.694
titin-cap (telethonin)	1.027 (0.604 to 1.83)	0.913
TNF receptor-associated factor 1	1.225 (1 to 1.942)	0.287
TNF receptor-associated factor 3	1.029 (0.827 to 1.206)	0.75
tocopherol (alpha) transfer protein (ataxia (Friedreich-like) with vitamin E deficiency)	0.922 (0.869 to 0.978)	0.401
toll-like receptor 1	0.978 (0.935 to 1.023)	0.705
toll-like receptor 2	1.278 (1.135 to 1.55)	0.128

toll-like receptor 4	1 (0.936 to 1.069)	0.998
transaldolase 1	1.203 (0.861 to 1.681)	0.678
transcobalamin I (vitamin B12 binding protein, R binder family)	0.817 (0.513 to 1.301)	0.739
transcobalamin II; macrocytic anemia	0.995 (0.977 to 1.029)	0.794
transcription elongation factor A (SII), 2	1.112 (1.084 to 1.141)	0.151
transcription elongation factor B (SIII), polypeptide 2 (18kDa, elongin B)	2.91	no replicates
transcription elongation factor B (SIII), polypeptide 3 (110kDa, elongin A)	1.15 (0.685 to 2.463)	0.642
transcription factor 1, hepatic; LF-B1, hepatic nuclear factor (HNF1), albumin proximal factor	0.902 (0.478 to 1.85)	0.736
transcription factor 19 (SC1)	1.08 (1.049 to 1.112)	0.231
transcription factor 2, hepatic; LF-B3; variant hepatic nuclear factor	1.032 (0.946 to 1.127)	0.778
transcription factor 21	0.963 (0.942 to 1)	0.185
transcription factor 4	0.963 (0.924 to 0.99)	0.219
transcription factor A, mitochondrial	2.931 (1.477 to 5.818)	0.361
transcription factor AP-2 beta (activating enhancer binding protein 2 beta)	1.118 (0.862 to 1.45)	0.741
transcription factor AP-2 gamma (activating enhancer binding protein 2 gamma)	1.054 (0.984 to 1.129)	0.582
transcription factor binding to IGHM enhancer 3	0.958 (0.591 to 1.614)	0.895
transcription termination factor, RNA polymerase II	1.073 (0.917 to 1.255)	0.733
transcriptional intermediary factor 1	1.01 (0.837 to 1.219)	0.967
transducin (beta)-like 1X-linked	0.117	no replicates
transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	1.456 (1.097 to 1.932)	0.411
transducin-like enhancer of split 2 (E(sp1) homolog, Drosophila)	0.939 (0.774 to 1.138)	0.797
transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)	1.278 (0.791 to 2.144)	0.484
transferrin receptor 2	1.046 (0.921 to 1.189)	0.783
transforming growth factor, beta 3	0.94 (0.595 to 1.484)	0.914
transforming, acidic coiled-coil containing protein 1	0.901 (0.458 to 1.636)	0.715
transgelin	1.392 (1.032 to 1.878)	0.468
transgelin 2	1.177 (1.118 to 1.256)	0.0413
transglutaminase 1 (K polypeptide epidermal type I, protein-glutamine-gamma-glutamyltransferase)	1.018 (0.874 to 1.233)	0.849
transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	0.908 (0.76 to 1.089)	0.247
transglutaminase 3 (E polypeptide, protein-glutamine-gamma-glutamyltransferase)	1.079 (0.969 to 1.202)	0.608
transient receptor potential cation channel, subfamily C, member 1	1.088 (0.988 to 1.199)	0.542
transient receptor potential cation channel, subfamily C, member 3	0.892 (0.874 to 0.91)	0.112
transient receptor potential cation channel, subfamily M, member 1	1.208 (0.939 to 1.553)	0.59
transient receptor potential cation channel, subfamily M, member 2	0.874 (0.555 to 1.342)	0.544
transition protein 1 (during histone to protamine replacement)	1.1 (0.878 to 1.642)	0.682
transition protein 2 (during histone to protamine replacement)	1.193 (0.843 to 1.745)	0.49
transketolase (Wernicke-Korsakoff syndrome)	0.754 (0.504 to 1.14)	0.297
translocase of inner mitochondrial membrane 8 homolog A (yeast)	0.968 (0.955 to 0.98)	0.241
translocated promoter region (to activated MET oncogene)	2.599	no replicates
transmembrane 4 superfamily member 3	1.585 (0.745 to 5.028)	0.514
transmembrane 4 superfamily member 4	1.14 (0.894 to 1.455)	0.685
transmembrane 4 superfamily member 6	0.834 (0.81 to 0.86)	0.104
transmembrane 4 superfamily member 7	1.299 (0.901 to 3.796)	0.39
transmembrane 7 superfamily member 1 (upregulated in kidney)	0.761 (0.749 to 0.773)	0.0371
transmembrane 7 superfamily member 2	1.109 (1.019 to 1.205)	0.435
transmembrane protease, serine 2	0.919 (0.906 to 0.932)	0.103
transmembrane protein 1	1.108 (1 to 1.361)	0.423
transmembrane protein with EGF-like and two follistatin-like domains 1	0.891 (0.658 to 1.162)	0.321
transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	0.907 (0.675 to 1.22)	0.798
transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	0.501 (0.177 to 0.869)	0.316
Treacher Collins-Franceschetti syndrome 1	1.198 (0.619 to 3.777)	0.615
trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in)	1.092 (0.806 to 1.367)	0.483
trefoil factor 2 (spasmolytic protein 1)	0.976 (0.608 to 1.567)	0.967
trefoil factor 3 (intestinal)	0.781 (0.526 to 0.971)	0.338
triosephosphate isomerase 1	0.987 (0.825 to 1.179)	0.952
tripartite motif-containing 26	0.909 (0.51 to 1.742)	0.756
tripartite motif-containing 3	0.829 (0.795 to 0.864)	0.139
tripeptidyl peptidase II	1.016 (0.837 to 1.233)	0.948
triple functional domain (PTPRF interacting)	0.813 (0.417 to 1.285)	0.606
trophinin	0.886 (0.574 to 1.258)	0.52
trophoblast glycoprotein	0.963 (0.893 to 1.038)	0.706
tropomodulin 1	1.312 (0.434 to 4.328)	0.604
tropomyosin 1 (alpha)	0.972 (0.792 to 1.412)	0.894
tropomyosin 2 (beta)	0.926 (0.576 to 1.319)	0.687
tropomyosin 4	0.947 (0.946 to 0.947)	0.00492

troponin C, slow	1.093 (1.055 to 1.115)	0.0371
troponin C2, fast	1.024 (0.984 to 1.083)	0.49
troponin I, cardiac	1.147 (1.141 to 1.152)	0.0224
troponin I, skeletal, fast	0.816 (0.741 to 0.898)	0.281
troponin I, skeletal, slow	0.833 (0.8 to 0.866)	0.135
troponin T1, skeletal, slow	1.039 (0.524 to 1.656)	0.849
troponin T2, cardiac	0.743 (0.574 to 0.913)	0.163
troponin T3, skeletal, fast	1.516 (1.066 to 3.003)	0.347
tryptase beta 1	0.865 (0.731 to 1.162)	0.431
tryptase, alpha	1.093 (0.764 to 1.565)	0.845
tryptophan 2,3-dioxygenase	1.08 (0.744 to 1.551)	0.75
tryptophan rich basic protein	0.875 (0.796 to 0.962)	0.394
tryptophanyl-tRNA synthetase	0.791 (0.529 to 1.218)	0.434
TTK protein kinase	0.992 (0.82 to 1.378)	0.965
Tu translation elongation factor, mitochondrial	0.942 (0.683 to 1.23)	0.641
tubby homolog (mouse)	0.725 (0.203 to 1.606)	0.524
tubby like protein 1	0.736 (0.193 to 1.343)	0.427
tubby like protein 2	1.17 (1.057 to 1.352)	0.169
tubby like protein 3	1.19 (1.077 to 1.316)	0.333
tuberous sclerosis 1	0.989 (0.769 to 1.273)	0.973
tubulin, alpha, ubiquitous	2.284 (0.659 to 7.915)	0.627
tubulin, beta polypeptide	0.611 (0.436 to 1.176)	0.271
tubulin, gamma 1	0.986 (0.812 to 1.112)	0.899
tubulin-specific chaperone a	0.725 (0.673 to 0.799)	0.0239
tubulin-specific chaperone c	0.757	no replicates
tubulin-specific chaperone d	0.862 (0.507 to 1.261)	0.64
tubulin-specific chaperone e	1.599 (0.565 to 6.608)	0.589
tumor necrosis factor (ligand) superfamily, member 10	1.099 (0.912 to 1.325)	0.702
tumor necrosis factor (ligand) superfamily, member 12	1.527 (1.026 to 3.149)	0.363
tumor necrosis factor (ligand) superfamily, member 13	1.292 (1.14 to 1.464)	0.289
tumor necrosis factor (ligand) superfamily, member 14	1.171 (0.552 to 1.992)	0.599
tumor necrosis factor (ligand) superfamily, member 4 (tax-transcriptionally activated glycoprotein 1, 34kDa)	0.962 (0.871 to 1.063)	0.767
tumor necrosis factor (ligand) superfamily, member 5 (hyper-IgM syndrome)	0.864 (0.712 to 1.047)	0.586
tumor necrosis factor (ligand) superfamily, member 6	1.211 (0.891 to 1.788)	0.45
tumor necrosis factor (ligand) superfamily, member 8	0.954 (0.724 to 1.844)	0.844
tumor necrosis factor (ligand) superfamily, member 9	0.898 (0.897 to 0.899)	0.008
tumor necrosis factor receptor superfamily, member 11a, activator of NFKB	0.856 (0.566 to 1.542)	0.659
tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	1.603 (1.113 to 2.309)	0.419
tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator)	0.568 (0.259 to 1.231)	0.336
tumor necrosis factor receptor superfamily, member 18	1.941 (1.875 to 2.01)	0.0334
tumor necrosis factor receptor superfamily, member 25	1.068 (0.778 to 1.466)	0.869
tumor necrosis factor receptor superfamily, member 4	0.814 (0.608 to 1.07)	0.122
tumor necrosis factor receptor superfamily, member 6b, decoy	1.04 (0.973 to 1.111)	0.661
tumor necrosis factor receptor superfamily, member 9	0.91 (0.825 to 1.002)	0.508
tumor necrosis factor, alpha-induced protein 3	1.2 (1.164 to 1.236)	0.104
tumor protein D52	1.19 (1.037 to 1.365)	0.426
tumor protein D52-like 1	1.149 (1.076 to 1.227)	0.282
tumor protein D52-like 2	0.903 (0.734 to 1.245)	0.594
tumor protein p53 binding protein, 1	1.22 (1.165 to 1.277)	0.143
tumor protein p73-like	0.689 (0.572 to 0.83)	0.295
tumor protein, translationally-controlled 1	1.736 (1.396 to 2.159)	0.24
tumor rejection antigen (gp96) 1	0.929 (0.665 to 1.24)	0.628
tumor suppressing subtransferable candidate 1	0.966 (0.625 to 1.413)	0.85
tumor suppressing subtransferable candidate 3	0.702	no replicates
tumor-associated calcium signal transducer 1	1.203 (0.676 to 1.875)	0.479
twist homolog 1 (acrocephalosyndactyly 3; Saethre-Chotzen syndrome) (Drosophila)	1.228 (0.925 to 1.841)	0.427
TXK tyrosine kinase	1.275	no replicates
TYRO protein tyrosine kinase binding protein	0.799 (0.458 to 1.181)	0.515
tyrosinase (oculocutaneous albinism IA)	1.272 (1.211 to 1.403)	0.0387
tyrosinase-related protein 1	1.066 (0.875 to 1.493)	0.743
tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide	1.132 (0.743 to 1.748)	0.5
tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	1.463	no replicates
tyrosine aminotransferase	0.975 (0.635 to 2.084)	0.908

tyrosine kinase 2	1.039 (0.926 to 1.166)	0.794
tyrosine kinase with immunoglobulin and epidermal growth factor homology domains	0.992 (0.695 to 1.583)	0.966
tyrosine kinase, non-receptor, 1	0.411 (0.0565 to 1.131)	0.465
tyrosylprotein sulfotransferase 1	1.361 (1.103 to 1.555)	0.101
tyrosylprotein sulfotransferase 2	1.178 (1.006 to 1.38)	0.488
tyrosyl-tRNA synthetase	1 (0.998 to 1.002)	0.984
ubiquinol-cytochrome c reductase core protein I	1.44 (1.091 to 1.901)	0.414
ubiquinol-cytochrome c reductase core protein II	0.981 (0.856 to 1.125)	0.912
ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	1.186 (0.323 to 2.169)	0.726
ubiquitin protein ligase E3A (human papilloma virus E6-associated protein, Angelman syndrome)	0.771 (0.762 to 0.78)	0.0286
ubiquitin specific protease 1	1.378 (1.054 to 2.041)	0.251
ubiquitin specific protease 11	1.438 (0.987 to 2.531)	0.334
ubiquitin specific protease 4 (proto-oncogene)	0.802 (0.355 to 1.362)	0.648
ubiquitin specific protease 5 (isopeptidase T)	0.887 (0.322 to 2.048)	0.805
ubiquitin specific protease 7 (herpes virus-associated)	0.917 (0.768 to 1.116)	0.51
ubiquitin-activating enzyme E1 (A1S9T and BN75 temperature sensitivity complementing)	0.714 (0.335 to 1.241)	0.308
ubiquitin-conjugating enzyme E2 variant 1	0.826 (0.511 to 1.121)	0.213
ubiquitin-conjugating enzyme E2B (RAD6 homolog)	1.386 (0.84 to 2.286)	0.632
ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast)	1.024 (0.97 to 1.08)	0.74
ubiquitin-conjugating enzyme E2E 1 (UBC4/5 homolog, yeast)	1.342 (1.109 to 1.624)	0.366
ubiquitin-conjugating enzyme E2G 2 (UBC7 homolog, yeast)	0.618 (0.249 to 1.371)	0.434
ubiquitin-conjugating enzyme E2H (UBC8 homolog, yeast)	1.318 (0.952 to 1.942)	0.0744
ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast)	1.135 (1.023 to 1.389)	0.339
ubiquitin-conjugating enzyme E2L 3	0.823 (0.62 to 1.092)	0.616
ubiquitin-like 1 (sentrin)	0.86 (0.563 to 1.56)	0.551
ubiquitin-like 3	1.222 (0.23 to 2.99)	0.751
ubiquitously-expressed transcript	1.119 (1.095 to 1.144)	0.121
UDP glycosyltransferase 1 family, polypeptide A6	1.027 (1.018 to 1.036)	0.211
UDP glycosyltransferase 2 family, polypeptide B10	0.978 (0.473 to 1.844)	0.941
UDP glycosyltransferase 2 family, polypeptide B7	1.05 (0.84 to 1.226)	0.636
UDP glycosyltransferase 8 (UDP-galactose ceramide galactosyltransferase)	0.928 (0.825 to 1.044)	0.641
UDP-Gal:betaGal beta 1,3-galactosyltransferase polypeptide 6	1.223 (0.938 to 1.636)	0.239
UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	1.228 (1.02 to 1.479)	0.468
UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 5	0.914 (0.843 to 0.99)	0.462
UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 1	0.896 (0.692 to 1.161)	0.487
UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 2	1.96 (1.217 to 3.156)	0.392
UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 3	0.907 (0.648 to 1.072)	0.454
UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 5	0.685 (0.5 to 0.937)	0.44
UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 6	1.074 (0.586 to 2.324)	0.821
UDP-glucose ceramide glucosyltransferase	1.118 (0.661 to 2.424)	0.803
UDP-glucose dehydrogenase	1.391 (0.988 to 1.758)	0.0731
UDP-N-acetyl-alpha-D-galactosamine:(N-acetylneuraminyl)-galactosylglucosylceramide N-acetylgalactosaminyltransferase (GalNAc-T)	1.162 (0.947 to 1.444)	0.123
UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GalNAc-T2)	1.374 (1.245 to 1.516)	0.191
UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3)	1.133 (0.822 to 1.475)	0.405
UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 4 (GalNAc-T4)	1.097 (0.898 to 1.35)	0.352
UDP-N-acteylglucosamine pyrophosphorylase 1	0.957 (0.926 to 0.99)	0.416
unc-5 homolog C (C. elegans)	0.818 (0.369 to 1.461)	0.538
unc-51-like kinase 1 (C. elegans)	1.01 (0.661 to 1.882)	0.956
uncoupling protein 2 (mitochondrial, proton carrier)	1.038 (0.757 to 1.422)	0.926
uncoupling protein 3 (mitochondrial, proton carrier)	1.029 (1.015 to 1.044)	0.286
undifferentiated embryonic cell transcription factor 1	1.625 (1.395 to 1.894)	0.194
upstream transcription factor 2, c-fos interacting	1.192	no replicates
uridine monophosphate synthetase (orotate phosphoribosyl transferase and orotidine-5'-decarboxylase)	1.483 (1.265 to 1.738)	0.244
uridine phosphorylase	1.202 (1.022 to 1.526)	0.27
urocortin	1.081 (0.912 to 1.282)	0.726
uroporphyrinogen decarboxylase	4.292	no replicates
uroporphyrinogen III synthase (congenital erythropoietic porphyria)	1.104 (0.915 to 1.479)	0.575
UV radiation resistance associated gene	1.402 (1.085 to 2.149)	0.123
vaccinia related kinase 1	0.891 (0.614 to 1.549)	0.56
vaculolar ATPase	0.777 (0.406 to 1.173)	0.523
v-akt murine thymoma viral oncogene homolog 2	0.949 (0.772 to 1.167)	0.843

vasoactive intestinal peptide	0.801 (0.461 to 1.149)	0.511
vasoactive intestinal peptide receptor 1	1.159 (0.581 to 2.622)	0.576
vasoactive intestinal peptide receptor 2	1.21 (0.713 to 2.514)	0.664
vasodilator-stimulated phosphoprotein	0.717 (0.537 to 0.906)	0.162
vav 2 oncogene	0.935 (0.413 to 2.312)	0.906
v-crk sarcoma virus CT10 oncogene homolog (avian)	0.986 (0.923 to 1.053)	0.865
v-crk sarcoma virus CT10 oncogene homolog (avian)-like	0.754 (0.378 to 1.561)	0.404
very low density lipoprotein receptor	0.885 (0.763 to 1.026)	0.561
Very very hypothetical protein RMSA-1	0.966 (0.949 to 0.984)	0.313
vesicle docking protein p115	1.137 (0.887 to 1.529)	0.237
vesicle-associated membrane protein 4	0.786 (0.513 to 1.256)	0.451
vesicle-associated membrane protein 8 (endobrevin)	1.378 (0.917 to 2.07)	0.575
v-ets erythroblastosis virus E26 oncogene like (avian)	1.368 (1.326 to 1.41)	0.0621
VGF nerve growth factor inducible	0.999 (0.861 to 1.146)	0.99
v-Ha-ras Harvey rat sarcoma viral oncogene homolog	0.631 (0.475 to 0.83)	0.0282
villin 2 (ezrin)	1.368 (0.929 to 3.129)	0.342
vimentin	1.669 (1.19 to 2)	0.0939
vinculin	1.752 (1.407 to 2.212)	0.00217
visinin-like 1	1.41 (0.483 to 3.427)	0.377
vitamin D (1,25- dihydroxyvitamin D3) receptor	0.861 (0.644 to 1.026)	0.261
vitelliform macular dystrophy (Best disease, bestrophin)	1.528 (0.926 to 3.246)	0.384
v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	1.322	no replicates
v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	0.755 (0.531 to 0.908)	0.101
v-maf musculoaponeurotic fibrosarcoma oncogene homolog G (avian)	1.129 (0.874 to 1.458)	0.719
v-myb myeloblastosis viral oncogene homolog (avian)	1.38 (0.883 to 2.206)	0.203
v-myc myelocytomatosis viral oncogene homolog 2 (avian)	1.286 (0.789 to 2.563)	0.301
voltage-dependent anion channel 1	0.942 (0.757 to 1.337)	0.768
voltage-dependent anion channel 2	0.86 (0.592 to 1.137)	0.519
von Hippel-Lindau binding protein 1	0.929 (0.751 to 1.159)	0.614
von Hippel-Lindau syndrome	1.166 (0.74 to 1.813)	0.372
von Willebrand factor	1.166 (0.82 to 1.504)	0.316
v-raf murine sarcoma 3611 viral oncogene homolog 1	1.027 (0.887 to 1.319)	0.851
v-ral simian leukemia viral oncogene homolog B (ras related; GTP binding protein)	0.839 (0.787 to 0.894)	0.221
v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	1.039 (0.955 to 1.132)	0.728
WEE1 homolog (S. pombe)	1.147 (0.973 to 1.328)	0.121
Werner syndrome	0.747 (0.619 to 0.902)	0.365
wingless-type MMTV integration site family, member 11	1.229 (0.815 to 1.982)	0.509
wingless-type MMTV integration site family, member 5A	1.238 (1.092 to 1.404)	0.338
wingless-type MMTV integration site family, member 7A	1.33 (1.039 to 1.508)	0.044
wingless-type MMTV integration site family, member 8B	1.407 (0.837 to 2.855)	0.45
Wiskott-Aldrich syndrome protein interacting protein	1.157 (1.151 to 1.162)	0.0219
xanthine dehydrogenase	0.737 (0.632 to 0.86)	0.299
Zic family member 1 (odd-paired homolog, Drosophila)	0.89 (0.858 to 0.923)	0.194
Zic family member 3 heterotaxy 1 (odd-paired homolog, Drosophila)	0.943 (0.371 to 1.522)	0.844
zinc finger protein 124 (HZF-16)	1.208 (1.158 to 1.261)	0.141
zinc finger protein 132 (clone pHZ-12)	1.215 (0.746 to 1.72)	0.52
zinc finger protein 133 (clone pHZ-13)	1.142 (0.67 to 2.378)	0.759
zinc finger protein 134 (clone pHZ-15)	1.111 (0.955 to 1.292)	0.614
zinc finger protein 135 (clone pHZ-17)	0.848 (0.747 to 0.955)	0.145
zinc finger protein 136 (clone pHZ-20)	1.457 (1.33 to 1.595)	0.151
zinc finger protein 137 (clone pHZ-30)	1.233 (0.925 to 1.643)	0.598
zinc finger protein 140 (clone pHZ-39)	1.794 (1.196 to 2.691)	0.386
zinc finger protein 141 (clone pHZ-44)	1.144 (0.831 to 2.147)	0.711
zinc finger protein 143 (clone pHZ-1)	1.291 (1.236 to 1.349)	0.107
zinc finger protein 151 (pHZ-67)	1.468 (0.825 to 3.02)	0.229
zinc finger protein 157 (HZF22)	1.002 (0.749 to 1.341)	0.996
zinc finger protein 165	0.865 (0.607 to 1.046)	0.499
zinc finger protein 174	1.239	no replicates
zinc finger protein 177	1.455 (0.748 to 4.164)	0.266
zinc finger protein 198	1.138 (0.846 to 1.531)	0.738
zinc finger protein 200	1.107 (0.997 to 1.23)	0.51
zinc finger protein 205	1.061 (0.72 to 1.599)	0.822
zinc finger protein 207	1.915	no replicates
zinc finger protein 297	0.98 (0.416 to 2.265)	0.971
zinc finger protein 345	1.005 (0.73 to 1.384)	0.99
zinc finger protein 35 (clone HF.10)	1.795 (1.499 to 2.149)	0.19
zinc finger protein 354A	0.976 (0.818 to 1.156)	0.832
zinc finger protein 36, C3H type-like 2	0.884 (0.861 to 0.907)	0.133
zinc finger protein 37 homolog (mouse)	1.311 (1.234 to 1.392)	0.14

zinc finger protein 42 (myeloid-specific retinoic acid- responsive)	1.147 (1.057 to 1.244)	0.341
zinc finger protein 43 (HTF6)	0.598 (0.408 to 0.876)	0.407
zinc finger protein 45 (a Kruppel-associated box (KRAB) domain polypeptide)	1.045 (0.609 to 1.5)	0.887
zinc finger protein 7 (KOX 4, clone HF.16)	1.097 (0.769 to 1.565)	0.837
zinc finger protein 74 (Cos52)	1.702 (0.938 to 3.089)	0.536
zinc finger protein 76 (expressed in testis)	1.43 (1.124 to 1.819)	0.377
zinc finger protein 84 (HPF2)	1.246 (0.738 to 1.598)	0.309
zinc finger protein 91 (HPF7, HTF10)	2.209 (1.33 to 3.668)	0.362
zinc finger protein, X-linked	1.058	no replicates
zinc finger protein, Y-linked	1.197 (0.954 to 1.501)	0.574
zona pellucida glycoprotein 2 (sperm receptor)	0.743 (0.369 to 2.567)	0.449
zyxin	0.963 (0.72 to 1.316)	0.85