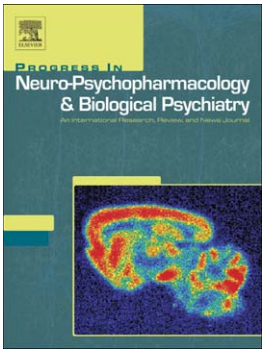

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Evidence that genetic variation in the oxytocin receptor (*OXTR*) gene influences social cognition in ADHD

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ABSTRACT:

Some children with ADHD also have social and communication difficulties similar to those seen in children with autistic spectrum disorders and this may be due to shared genetic liability. As the oxytocin receptor (*OXTR*) gene has been implicated in social cognition and autistic spectrum disorders, this study investigated whether *OXTR* polymorphisms previously implicated in autism were associated with ADHD and whether they influenced *OXTR* mRNA expression in 27 normal human amygdala brain samples. The family based association sample consisted of 450 DSM-IV diagnosed ADHD probands and their parents. Although there was no association with the ADHD phenotype, an association with social cognitive impairments in a subset of the ADHD probands (N= 112) was found for SNP rs53576 (F=5.24, p = 0.007) with post hoc tests demonstrating that the AA genotype was associated with better social ability compared to the AG genotype. Additionally, significant association was also found for rs13316193 (F=3.09, p = 0.05) with post hoc tests demonstrating that the CC genotype was significantly associated with poorer social ability than the TT genotype. No significant association between genotype and *OXTR* mRNA expression was found. This study supports previous evidence that the *OXTR* gene is implicated in social cognition.

KEYWORDS:

ADHD; OXTR; Association; Social Cognition; Human post mortem brain tissue

ABBREVIATIONS:

ADHD, Attention Deficit Hyperactivity Disorder; β-2M, beta 2-Microglobulin; CEPH, Centre d'Etude du Polymorphisme Humain; CTRS, Conners Teacher Rating Scale; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders ; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; LD, linkage disequilibrium; OD, Optical density; ODD, oppositional defiant disorder; OXTR, oxytocin receptor; PMI, post mortem interval; SCDC, Social and Communication Disorders Checklist; SNP's, single nucleotide polymorphisms; TDT, transmission disequilibrium test

INTRODUCTION:

Attention Deficit Hyperactivity Disorder (ADHD) is one of the most prevalent and heritable childhood psychiatric disorders (Asherson, 2004), affecting an estimated 5% of school aged children (Polanczyk and Rohde, 2007). A proportion of children with ADHD also have social and communication difficulties similar to those seen in the autistic spectrum disorders (Pellham and Bender, 1982). These behaviours include use of non verbal behaviours such as eye gaze, gesture and body postures, a lack of seeking to share, failure to develop appropriate peer relationships, lack of social reciprocity, repetitive or stereotyped language and lack of imaginative or imitative play. Some of these apparent similarities are likely the result of overlap of behaviours, which on first sight appear similar but probably stem from different origins e.g failing to read social cues versus impulsive behaviour that is socially inappropriate. However, this explanation is less likely for other social communication symptoms: some children with ADHD have significant difficulties with eye gaze, use of gesture, imitative and imaginative play and don't offer to share (Mulligan et al, 2009).

Mulligan et al (2009) also provided evidence that the overlap of some autism and ADHD symptoms that they found in ADHD probands was not due to measurement artifact. Of particular interest are recent findings demonstrating that autistic and ADHD traits share some underlying genetic liability (Ronald et al. , 2008, Mulligan et al. , 2009) suggesting that the two disorders may share some common genetic aetiology.

Evidence from animal and human studies implicates the oxytocin system in the development of social cognition. Many animal studies have demonstrated that oxytocin has a role in social bonding (Keverne and Curley, 2004, Winslow and Insel, 2002) and male mice with a targeted forebrain knockout of the oxytocin receptor gene (*OXTR*) exhibit social impairment (Lee et al. , 2008). As predicted from these animal studies, oxytocin plasma levels have been found to be decreased in some prepubertal individuals with autism compared to non autistic controls (Modahl et al. , 1998) and oxytocin administration decreases repetitious behaviour in some autistic individuals (Hollander et al. , 2007). In a recent study Domes et al. (2007b) showed that intranasally administered oxytocin improved the ability to infer the mental state of others from social cues of the eye region. Oxytocin administration in normal volunteers has also been shown to increase eye-eye gaze time and emotional recognition, which is believed to underlie the deficits in social communication (Guastella et al. , 2008). Recently oxytocin nasal spray has also been shown to improve emotion recognition in young people diagnosed with autism spectrum disorders (Guastella et al. , 2009) as well as promote social approach and social comprehension in individuals with autism (Andari et al. , 2010). The amygdala plays an important role in emotional processing (Davis and Whalen, 2001) including

recognition of facial emotions (Adolphs, 2002). The oxytocin receptor is highly concentrated in the amygdala (Huber et al. , 2005, Veinante and Freund-Mercier, 1997) and oxytocin has shown substantial binding in the region, as measured by receptor autoradiography and in situ hybridisation (Bale et al. , 2001, Huber et al. , 2005, Landgraf and Neumann, 2004). It is postulated that oxytocin reduces activation of the amygdala, inhibiting social anxiety, indicating a neural mechanism for the effects of oxytocin in social cognition in humans (Domes et al. , 2007a).

Previous studies have reported evidence that genetic variation in the *OXTR* gene may be implicated in autism susceptibility. Two genome-wide linkage scans in autism provided evidence for suggestive linkage at 3p25, a region of the genome that includes the *OXTR* gene (Lauritsen et al. , 2006, McCauley et al. , 2005). The *OXTR* gene spans approximately 19 kilobases (kb) and contains four exons and three introns. Intron 3 accounts for approximately 14 kb of the 19 kb. Wu et al (2005) examined 4 single nucleotide polymorphisms (SNP's) in a sample of Chinese individuals with autism and found association with the Intron 3 SNP's, rs2254298 and rs53576. Subsequently Jacob et al (2007) found association in a Caucasian population with rs2254298 reported by Wu et al (2005), but on the contrasting allele. In a recent case control study, Lui et al (2010) also found evidence for association of rs2254298 and rs2268491with autistic spectrum disorder in a Japanese population, which remained significant after Bonferroni correction. Their finding for rs2254298 was with the same A allele as the Chinese population study of Wu et al (2005). Lerer et al (2007) investigated 18 SNPs in a sample of Israeli individuals with autism and found several to be nominally associated, concentrated mostly within intron 3. A haplotype containing the A allele of rs2254398 was found to be protective, in keeping with the

Jacob et al (2007) study. Both family based and case control analyses in the Lui et al study suggested the A allele of rs53576 may also be involved in autism susceptibility but this only just reached statistical significance (p=0.05) in the case control analysis and disappeared upon correction for multiple testing. Wermter et al (2010) recently demonstrated a significant association at p<0.05 with rs2270465 in 100 autistic spectrum cases but this finding did not survive correction for multiple testing. They also found overtransmission of several haplotypes containing the A allele of rs53576, in keeping with both the Wu et al (2005) and Lui et al (2010) studies.

A recent investigation of the functionality of SNP's previously associated with autism provided evidence that rs237885 and rs13316193 influenced *OXTR* mRNA expression in human brain tissue, and that rs237895 and rs237897 explained the most variance seen in an allelic expression imbalance assay in a lymphoblastoid cell line (L Gallagher, personal communication). Additionally, Gregory et al (2009) recently reported a case of autism associated with a heterozygous deletion of the *OXTR* gene. They also identified a single CpG dinucleotide within the known *OXTR* control region overlapping exons 1-3, which showed a significant increase in methylation in peripheral blood mononuclear cells and temporal cortex tissue of autism cases compared to controls. As predicted, the autism cases also demonstrated lower mRNA *OXTR* expression compared to controls

Given the presence of social cognition deficits in some children with ADHD and the shared genetic liability between ADHD and autistic symptoms reported by Mulligan et al (2009), this study investigated whether some of the SNP's in the *OXTR* gene previously implicated in autism susceptibility are also associated with either the

ADHD phenotype or social cognition traits in ADHD probands. We also investigated whether risk alleles of SNP's associated with autism reduced *OXTR* mRNA levels in human post mortem amygdala brain tissue.

METHODS:

Association Sample

For this study 450 ADHD probands (inclusive of twelve affected siblings) and their parents were recruited from several child psychiatry clinics in the UK (N=183) and Ireland (N=267), following approval from the appropriate research ethics committees. After complete description of the study to the subjects, written informed consent was obtained from parents and children (some younger children gave assent). For 380 of the probands from the UK and Ireland, parents were interviewed by trained psychiatrists or psychologists employing the Child and Adolescent Psychiatric Assessment (CAPA) (Angold et al. , 1995). Consistent interview procedures were employed across the two centres with researchers from each centre receiving a common training in the use of the CAPA. Inter-rater reliability kappa coefficients were calculated for ADHD subtype diagnoses (κ=0.82; CI = 0.71-0.94). The remaining 70 UK probands were interviewed with the parent version of the K-SADS interview (Kaufman et al. , 1997) by a trained psychologist.

In addition teacher ratings were obtained for children by the Conners Teacher Rating Scale (CTRS) (Conners, 1995). This was to confirm that symptoms met the criterion of pervasiveness. Established cut-off points for possible and likely ADHD caseness on the CTRS were adhered to i.e. a T score above 55 was required. All 450 probands were white and born in the UK or Ireland (Age range 4-16 years). The sample was predominantly male (90.1%) with no significant difference in sex ratio between the

two study recruitment centres. All probands fulfilled DSM-IV diagnostic criteria for Attention Deficit Hyperactivity Disorder (ADHD). Of these N=42 (9.3%) had the inattentive subtype, and N=38 (8.4%) had the hyperactive impulsive subtype, the remainder had combined subtype (82.3%). Children with an IQ below 70, or significant medical conditions such as epilepsy were excluded. Specifically, children with diagnosed autism, atypical autism or Aspergers syndrome were excluded as were any children who required further assessment of possible autistic spectrum disorder after the research interview procedure used in this study. Two hundred and eleven children (46.9%) had comorbid oppositional defiant disorder (ODD) and 69 children (15.3%) fulfilled criteria for comorbid conduct disorder. Frequencies of subtype and comorbidity were similar across the two recruitment centres. The social cognition phenotype was assessed by the Social and Communication Disorders Checklist completed by parents. The SCDC is a 12 item scale with high reliability and validity and is an efficient first-level screening questionnaire for autistic traits (Skuse et al. , 2005) (Appendix I). Each item can be scored between 0 and 2 with the total questionnaire score ranging from 0-24. The domains of content of the questions comprise social reciprocity, non-verbal skills, pragmatic language usage and functional impairment. In Skuse's original publication, the mean SCDC score for a group of children with autism and atypical autism was 16.6 (SD=5.7), psychiatric clinic control children mean score was 13.0 (SD=6.1) and normal population control children mean score was 2.9 (SD=4.0).

Human Post-Mortem Brain Sample

Twenty-seven samples (20 male, 7 female) of human post-mortem amygdala were obtained from the Medical Research Council (MRC), Sudden Death Brain and Tissue

Bank, Edinburgh prior to this study. Deceased individuals were aged 16-70 (mean age = 42.7). The mean post mortem interval (PMI) was 54 hours (SD = 18.0). However, since sample optical density (OD_{260}/OD_{280} ratios) and pH are regarded as more relevant indicators of RNA purity and integrity, than PMI or duration of agonal state per se (e.g. Bahn et al. , 2001; Harrison et al. , 2003), these were measured. Optical density (OD) was measured by a NanoVue (GE Health Care) and samples ranged from 1.6- 2.1. The pH of samples ranged from 5.7-6.7. RNA and DNA extraction was carried out using the MELT ^{TM} Total Nucleic Acid Isolation System (Applied Biosystem,^{AM} Foster City, 1983) The cDNA was synthesised with Reverse Transcriptase-mediated Polymerase Chain Reaction (RT-PCR) using the AffinityScript ^{TM} QPCR cDNA Synthesis Kit (Stratagene, CA 600559) on the Mx3005P machine (Stratagene, CA).

Genotyping

For association study samples, high molecular weight genomic DNA was extracted from whole blood (Wizard Genomic DNA purification A1620, Promega W1), cheek swab (Freeman et al. , 2003) or Oragene saliva collection systems (DNA Genotek, Ontario) according to standard procedures. Many of the SNP's across the *OXTR* gene are in high LD with each other and SNP's were chosen taking this into account so as to limit genotyping redundancy. Five SNP's (4 in Intron 3 and 1 in the 3'UTR) (see Table 1) were chosen based on previous findings: rs237885, rs13316193 and rs237995 were recently demonstrated to have functional consequences on *OXTR* mRNA (L Gallagher, personal communication); rs13316193 in addition to rs6770632 was also in several significant haplotypes associated with autistic spectrum disorder in Lerer et al (2007); rs53576 has been previously associated with autism with several

positive replications. Pre-designed SNP genotyping assays were obtained from Applied Biosystems for use on the Stratagene Mx3005P real-time PCR machine (Stratagene, CA) following standardised protocols provided with the assays.

mRNA Expression

The *OXTR* primers and probe sequences were acquired from Applied Biosystems (Foster City, CA). The probe was FAM labelled and designed based on sequences spanning across Exons 1 and 2 of the gene (Hs01041213_m1). Endogenous control gene assays were human GAPDH and human β-2M (Applied Biosystems, Foster City, CA). Quantitative PCR was performed in triplicate for each sample on a Stratagene Mx3005P using a standard protocol. The expression data produced were analysed and converted into threshold cycle values (Ct values) using the software program MxPro Version 4.0 (Stratagene, CA).

Statistical Analyses

For the association studies, TDT analysis for each sample was carried out using the programme software UNPHASED 3.1 (Dudbridge, 2003). Evidence for association was set at the 0.05 significance level. For the mRNA expression studies, mean values were obtained from the triplicate Ct values for each probe per sample. The target *OXTR* mRNA expression was normalised to endogenous reference genes (β-2M and GAPDH) to generate a ΔCt value. The relationship between the ΔCt values (*OXTR* mRNA relative expression) and the different genotype groups were analysed by ANOVA with Tukey post-hoc analyses in the analytical programme SPSS Data Editor Version 17.0. All p values are reported as uncorrected.

RESULTS:

ADHD Association Study Results

There were no Mendelian inheritance errors detected by Haploview (Barrett et al. , 2005). TDT analysis for the five SNP markers within the *OXTR* gene genotyped in an ADHD family-based sample did not demonstrate significant association of the gene with ADHD (Table 1). There was minimal linkage disequilibrium (LD) between the genotyped markers as determined by Haploview (Barrett et al. , 2005) with all marker pairs having an r^2 <0.2, except between rs53576 and rs237895 (r^2 = 0.43). TDT analysis for pair-wise haplotypes between all markers showed no significant associations.

Insert Table 1 here

Social Cognitive Score Association results

SCDC scores for 119 of the UK ADHD probands were available for analysis by genotype group. 7 extreme outliers were removed, leaving 112 probands (92.86% male) for analysis. There was no statistically significant difference in terms of number of ADHD or ODD symptoms or gender between these 112 probands and the remainder of the 450 probands for whom no data was available. The mean SCDC score was 18.17 (SD 4.58). Scores were normally distributed. Genotype frequencies in the parents of the 112 cases did not depart from Hardy Weinberg equilibrium: rs237895 p=0.47, rs53576 p=0.4, rs13316193 p=1, rs237885 p=1, rs6770632 p=0.68. Potential confounders between SCDC scores and genotype, such as ADHD symptoms were investigated by correlational analysis. Hyperactive impulsive DSM IV symptom count was weakly, but significantly correlated with SCDC scores (r = 0.23, p = 0.02).

Given the very weak correlation, and therefore probable lack of biological relevance, analyses were initially performed without this covariate. Results are shown in Table 2. For rs13316193, the CC genotype was significantly associated with poorer social cognitive ability than the TT genotype (Tukey's p = 0.04) and for rs53576, the AA genotype was associated with better social cognitive ability compared to the AG genotype (Tukey's p = 0.008). The analysis was rerun with the covariate of hyperactivity/ impulsivity score and the SNP rs53576 finding remained significant (F=4.57, p = 0.01)

Insert Table 2 here

OXTR mRNA Expression

Gene expression of the *OXTR* gene in 27 human post-mortem amygdala samples was approximated by the measurement of mRNA levels via quantitative PCR. *OXTR* mRNA expression levels were normally distributed by Kolomogorov Smirnov tests, and co-factors such as OD ratio, pH, post mortem interval and sex were not correlated with *OXTR* mRNA and therefore not included as covariates in further analyses. There were no batch effects for different Quantitative-PCR runs. ANOVA demonstrated no significant difference in mRNA expression by genotype group for any of the SNP's (data not shown).

DISCUSSION:

The diagnostic overlap and similarities between ADHD and autistic spectrum disorders has long been acknowledged (Pellham and Bender, 1982) but has recently received more attention. Current evidence indicates that this overlap is due partly to shared genetic factors and is not merely the result of measurement artifact (Mulligan et al, 2009). Children with ADHD have significant difficulties in social interactions and communication (Reiersen et al. , 2007, Santosh and Mijovic, 2004) which are similar to those seen in pervasive developmental disorders and exceed that which are found in control subjects (Hattori et al. , 2006). Significant correlations between ADHD and autistic traits have been demonstrated. In a community twin sample, Ronald et al, (2008) reported that between 72-96% of the phenotypic correlation of autistic and ADHD traits were explained by genetic influences. Mulligan et al (2009) recently demonstrated in a large sibling study that children with ADHD (who did not fulfil diagnostic criteria for an autistic spectrum disorder) had more autistic traits compared to their non-ADHD sibs or controls and that these traits were partly independent of symptoms of ADHD. They reported the percentage of phenotypic correlation due to shared familial influence to be 56% in males, (it could not be calculated for females) again suggesting some common underlying aetiology to both groups of symptoms.

Given the evidence for shared genetic liability of ADHD and autistic symptoms this study investigated whether genetic variation in the *OXTR* gene which has previously been associated with autism and social behaviours, may also be associated with ADHD. Although there was no evidence for involvement of the *OXTR* gene in ADHD symptoms per se, we did find preliminary evidence that it may be involved in the

social cognitive deficits seen in some ADHD children. The G allele of rs53576 and the C allele of rs13316193, which were associated with poorer social ability are the same alleles that were overtransmitted (albeit not statistically significantly) to ADHD offspring. These results should be interpreted with caution as they have not been corrected for multiple testing. For the specific SCDC analysis, the finding for rs53576 would remain significant if corrected for the five tests performed (p=0.035) but if corrected for all the analyses presented here then it would no longer reach statistical significance.

Wu et al (2005) reported evidence that the A allele of rs53576 was associated with autism in a Chinese Han population, both as a single marker and within several significantly associated haplotypes. More recently Lui et al (2010) also found weak association of the A allele with autistic spectrum disorder in a Japanese population. In our study, we also found evidence that rs53576 is associated with social cognitive deficits in ADHD children, but the A allele was associated with better social cognitive ability. The A allele frequencies for each population are similar (Wu et al study = 0.662; Lui et al study = 0.667; this study = 0.711) and the difference may reflect different haplotype structure between our European population and the Chinese population. This is supported by data from the HapMap project which demonstrates a considerably different haplotype structure across the *OXTR* gene between the Chinese Han and Japanese samples compared to the European CEPH samples. This suggests that rs53576 is not the true functional variant but that its two alleles have arisen on different haplotypes in different populations, but still in LD with the true functional variant in those different populations. A recent study by Wermter et al, (2010) provides evidence that possibly contradicts this explanation as they demonstrated

overtransmission of several haplotypes containing the A allele of rs53576 to autistic individuals of European ancestry, although the single marker analysis was not significant. However this was a small study in 100 cases where no finding survived correction for multiple testing. It therefore remains possible that the A allele is 'tagging' a functional variant associated with autism in Asian populations, whereas in Caucasian populations, the G allele is in LD with the functional variant. However, studies of other empathy related phenotypes have also found significant associations with poorer empathy and the A allele of rs53576. One study of Caucasian mothers demonstrated that those with the AA or AG genotype of rs53576 showed lower levels of sensitive responsiveness (which presupposes awareness of and empathy with children's needs) towards their toddlers (Bakermans-Kranenburg and van Ijzendoorn, 2008) and another recent study also found that individuals with one or two copies of the A allele exhibited lower behavioural and dispositional empathy (Rodrigues et al. , 2009).

Rs53576 is located in Intron 3, a region previously implicated in autism. Lerer et al (2007) found evidence for several single SNP and haplotype associations across the *OXTR* gene, with the most significant haplotype ($p = 0.00005$) spanning 5 SNP's across the region of Intron 3 containing rs53576. Additionally, rs2254298 which was also significantly associated in the Wu et al study (2005) is also within the region that this haplotype spans providing converging evidence that this region may be implicated in social cognitive deficits. Lerer et al (2007) speculated that their significant SNP's and haplotypes were in LD with a critical splice site polymorphism and Mizumoto et al (1997) reported that a genomic element in intron 3 may be involved in transcriptional suppression perhaps by altering methylation sites.

Subsequently, two regions of the *OXTR* gene (one overlapping exons 1, 2 and 3, and one in intron 3) have been shown to be methylated and associated with differential expression of the gene in liver and myometrium (Kimura et al. , 2003, Kusui et al. , 2001). Rs53576 in intron 3 lies 2.8kb from this methylated region and 4.6kb from the exon 1-3 methylated region so is unlikely to be directly involved in this process, although may be in LD with a relevant functional variant. Gregory et al (2009) identified a single CpG dinucleotide within the known *OXTR* control region overlapping exons 1-3, which showed a significant increase in methylation in peripheral blood mononuclear cells and temporal cortex tissue of autism cases compared to controls, providing evidence that aberrant methylation of the *OXTR* gene may contribute to autism.

We also investigated whether the SNP's genotyped for this study, influenced *OXTR* mRNA expression in human amygdala brain samples but found no association. The use of post mortem brain tissue has several advantages over *in vitro* techniques. The endogenous promoters and all other gene sequences etc needed for gene regulation are present in the tissue and this arguably provides a more relevant paradigm than cell lines and reporter vector systems, which do not represent normal tissue. However, brain tissue is composed of a variety of cell types such that mRNA expression levels of genes may vary between samples as a result of differences in cell composition of the particular piece of tissue used. Additionally, the tissue employed for this study was from 'normal' individuals with no known history of psychiatric disorder (although this can not be ruled out). We cannot rule out the possibility of a Type II error as our sample size was small but further investigation using larger samples and brain tissue from individuals with autism is warranted.

In summary, this study supports previous evidence that genetic variation in the *OXTR* gene influences social cognition and that this association but may be found in other diagnostic groups which display some impairment in social communication. However, replication in larger independent samples of ADHD individuals with social cognitive phenotypic information is needed. The recent evidence that oxytocin improves emotion recognition and social approach in people with autistic spectrum disorders (Guastella et al., 2009; Andari et al, 2010) may have implications for treating these deficits in individuals with ADHD and other diagnoses with social cognitive deficits.

APPENDIX 1

Social and Communication Disorders Checklist (Skuse et al, 2005)

1. Not aware of other people's feelings
2. Does not realise when others are upset or angry
3. Does not notice the effect of his/her behaviour on other members of the family
4. Behaviour often disrupts family life
5. Very demanding of other people's time
6. Difficult to reason with when upset
7. Does not seem to understand social skills, e.g. persistently interrupts conversations
8. Does not pick up on body language
9. Does not appear to understand how to behave when out (e.g. in shops, or other people's homes)
10. Does not realise if s/he offends people with her/his behaviour
11. Does not respond when told to do something
12. Cannot follow a command unless it is carefully worded

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Table I: TDT Analysis of *OXTR* gene SNP's

SNP	Chr Location	Gene Position	SNP Alleles	HW P value	% Genotyped	MAF (Allele)	P value	Over-transmitted Allele	T	NT	OR	95% CI
rs6770632	8733724	3'UTR	A/C	0.59	98.8	0.243 (A)	0.51	C	98	89	1.21	0.81-1.82
rs237885	8735543	Intron 3	G/T	1.00	95.7	0.469 (T)	0.69	T	119	113	1.11	0.77-1.59
rs13316193	8742743	Intron 3	C/T	0.16	95.3	0.348 (C)	0.72	C	101	96	1.11	0.75-1.64
rs53576	8744371	Intron 3	A/G	0.95	98.0	0.289 (A)	0.18	G	108	89	1.47	0.99-2.18
rs237895	8747423	Intron 3	C/T	0.40	96.8	0.403 (T)	0.64	C	116	109	1.13	0.78-1.64

Legend: Chr – Chromosome; HW – Hardy-Weinberg; MAF – Minor Allele Frequency; T – Transmitted; NT – Non-Transmitted; OR – Odds Ratio; CI – Confidence Interval
Odd Ratios and Confidence Intervals calculated using http://www.hutchon.net/ConfidORnulhypo.htm

Table II: ANOVA results for SCDC score by genotype.

SNP	Mean SCDC score (SD)	F	p
rs6770632 (N)			
AA (10)	19.10 (3.14)		
AC (38)	17.32 (5.11)	0.94	0.39
CC (60)	18.43 (4.48)		
rs237885 (N)			
GG (29)	19.00 (3.11)		
GT (65)	18.11 (4.99)	0.90	0.41
TT (16)	17.12 (4.92)		
rs13316193 (N)			
CC (14)	20.71 (2.23)		
CT (53)	18.02 (5.19)	3.09	0.05
TT (42)	17.26 (4.10)		
rs53576 (N)			
AA (8)	14.25 (4.53)		
AG (41)	19.44 (4.18)	5.24	0.007
GG (61)	17.67 (4.53)		
rs237895 (N)			
CC (47)	17.83 (4.07)		
CT (44)	18.52 (4.96)	0.86	0.43
TT (14)	16.71 (5.15)		