

**Investigation of retinal thickness using
optical coherence tomography: a
prospective phenotype study of patients
with inherited ataxia**

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Summary

Inherited cerebellar ataxias (CA) are clinically and genetically heterogeneous disorders. A diverse range of ophthalmic abnormalities have been reported in different subtypes of CA and, although visual symptoms are not always recognised, both afferent and efferent pathways can be affected. Abnormal retinal nerve fibre layer (RNFL) thickness to a degree measurable by optical coherence tomography (OCT) has been documented in common trinucleotide repeat disorders and in association with *SACS* (Sacsin Molecular Chaperone) gene mutations. Data on RNFL findings in other non-Friedreich's ataxia (FRDA) cohorts are scarce and little is known about retinal changes over time in CA.

My main hypothesis was that retinal changes in inherited ataxias are more common than previously thought, that OCT may play a role in distinguishing different types of ataxias, and that RNFL structural changes have a functional correlate, and thus, RNFL thickness measured with OCT has the potential to become a useful biomarker in CA.

The specific aims of the study were to characterise the clinical phenotypes and, using OCT, retinal findings associated with different types of inherited ataxias; to determine the pattern of retinal changes and their association with disease duration and functional disability quantified with the Scale for the Assessment and Rating of Ataxia (SARA) and to determine if retinal changes are evident over time.

In order to test this hypothesis, I performed an observational phenotype study of 131 patients with different types of determined or suspected genetic ataxia, and 7 asymptomatic first-degree relatives of affected individuals. Clinical assessment using a standardised approach, including detailed neurological examination was performed to characterise the phenotype of rare ataxic syndromes. Best-corrected visual acuity was measured using Snellen chart and

OCT was performed to document retinal findings at baseline and, when possible, at an interval to investigate if retinal changes are evident over time. Functional disability was assessed using SARA score and correlations with OCT measurements were assessed.

Throughout the course of the study, significant and most marked average RNFL thinning with predominant superior quadrant involvement was found in the FRDA group. Although the average RNFL thickness in the largest *SPG7*-associated spastic ataxia cohort subjected to OCT to date was not statistically different from controls, the temporal RNFL thickness was reduced in the patient group ($p < 0.05$). A distinct thickening of RNFL was found in individuals with ARSACS. Previously not reported optic neuropathy was found in *DDHD2*-, *ANO10*-, and *SAMD9L*-associated phenotypes, while no RNFL changes were detected in individuals with AOA1, CANVAS, *AIFM1*- and *MT-ATP6*-related disease. As expected, among the group of autosomal dominant CA (ADCA), retinal abnormalities were found in SCA7, contrary to SCA1, 2, 3, 6, 14, and EA2. There was a correlation between RNFL thickness and SARA score in the FRDA group, but no association was found in the *SPG7*-associated cases. Follow-up evaluation showed significant decline in most OCT parameters in FRDA as well as in average and temporal RNFL thickness measurements in the *SPG7* cohort simultaneously with disability progression, while a tendency to progressive RNFL thinning was documented in SCA1, but not in SCA3. In contrast, a tendency to increased macular and foveal thickness over time was seen in ARSACS, but average RNFL thickness or disease progression was not observed.

In conclusion, optic neuropathy is common in various subtypes of ataxia despite their clinical heterogeneity even in the absence of decreased visual acuity. Characteristic retinal changes, found predominantly in spastic ataxia phenotypes, indicate that OCT is a sensitive marker for distinguishing certain genetic ataxias and suggest that this cost-effective technique should be considered part of the routine evaluation in individuals with CA. The

longitudinal OCT data showed significant progression rates in some genetic ataxias and imply that OCT has the potential to become a quantifiable biomarker in future therapeutic trials.

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I would also like to acknowledge my friends whose interest in my research was very beneficial.

And finally, I would like to thank all the patients and healthy volunteers who agreed to participate in this study.

F.A. Wrong With Me

A poem by Anita Gracey [1] (Used with permission)

My veins carry a scar
genes cradle a slow deceit
visible in my mobility
my clumsy movements
slurred speech.

Father on his knees for a cure
mother lit candles in Lourdes
my doctor sweats over books
I scream quietly
shrug and plough on.

What's wrong with you?
Is the question, frequently asked
Freidreich's Ataxia I'd reply
Free wha? As eyes would glaze
Truth taught me to lie.

In my reckless teens to amuse
I'd freefall lie various accidents
show-jumping, hang-gliding or rock-climbing
teeter in bone-shaking detail
eyes would widen, jaws slacken.

My 20's I said motorbike accident
all that leather and speed
machine throbbing between my thighs
until the response - what type of bike was it?
tattoos crept from tell-tale biker jacket.

In my 30's hoping to deflate interrogation
I'd answer I've a cold.
There'd be flustered gesturing
to my wheels - No I mean that.
Oh I can't walk I'd add.

Nowadays there is beauty in my scar
and I say I've an impairment
it is the world around me which is 'wrong'.
I only whisper lies.

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Abbreviations

ACMG	American College of Medical Genetics guidelines
AD	Autosomal dominant
ADLQ	Activities of Daily Living Questionnaire
AIFM1	Apoptosis-inducing factor, mitochondria-associated-1
AR	Autosomal recessive
ARSACS	Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay
A-T	Ataxia Telangiectasia
ATP	Adenosine triphosphate
ATXN	Ataxin
AOA	Ataxia with oculomotor apraxia
BCVA	Best corrected visual acuity
CA	Cerebellar ataxia
CANVAS	Cerebellar ataxia with neuropathy and vestibular areflexia syndrome
CF	Count Fingers
CMT	Charcot-Marie-Tooth
CMTNS2	Charcot-Marie-Tooth Neuropathy Score 2
CTS	Carpal tunnel syndrome
DOA	Dominant optic atrophy
EA	Episodic ataxia
ETDRS	Early Treatment Diabetic Retinopathy Study grid
FARS	Friedreich's Ataxia Rating Scale
FRDA	Friedreich's ataxia

HIT	Head Impulse Test
HM	Hand movements
HSP	Hereditary spastic paraplegia
ICARS	International Cooperative Ataxia Rating Scale
ISCED	International Standard Classification of Education
LGN	Lateral geniculate nucleus
LHON	Leber's hereditary optic neuropathy
LogMAR	Logarithm of the minimal angle of resolution
MELAS	Mitochondrial Encephalomyopathy Lactic Acidosis Stroke-like episodes syndrome
MLPA	Multiplex ligation-dependent probe amplification
MoCA	Montreal Cognitive Assessment
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSA	Multiple system atrophy
MSA-C	Multiple system atrophy, cerebellar type
NAC	National Ataxia Clinic
NARP	Neuropathy, ataxia, and retinitis pigmentosa
NARU	Neurology Assessment and Research Unit
NCS	Nerve conduction studies
NGS	Next-generation sequencing
NMO	Neuromyelitis optica
OMA	Oculomotor apraxia
OCT	Optical coherence tomography
PD	Parkinson disease

RNFL	Retinal nerve fibre layer
RGC	Retinal ganglion cells
SARA	Scale for the Assessment and Rating of Ataxia
SCA	Spinocerebellar ataxia
SCAFI	Spinocerebellar Ataxia Functional Index
SD	Standard deviation
SD-OCT	Spectral-domain optical coherence tomography
SEMD	Spondyloepimetaphyseal dysplasia
SPG7	Spastic Paraplegiatype 7 gene
SPRS	Spastic Paraplegia Rating Scale
SWJ	Square wave jerks
TCC	Thin corpus callosum
TD-OCT	Time-domain optical coherence tomography
TUH	Tallaght University Hospital
VA	Visual acuity
VOR	Vestibulo-ocular reflex
VUS	Variant of uncertain significance
WES	Whole-exome sequencing
WGS	Whole-genome sequencing

Chapter 1: Introduction to optical coherence tomography in inherited ataxia

1.1 Cerebellar ataxias

The word ataxia is derived from the Greek, α - meaning “without” and -τάξις meaning “order”. A patient with ataxia therefore demonstrates lack of coordination while performing voluntary movements, which may appear as clumsiness, inaccuracy, or instability.

Cerebellar ataxias (CA) are a diverse group of rare disorders with a variety of underlying aetiologies that can be broadly divided into two main groups: genetic or acquired. However, for a proportion of patients, who present with adult onset CA in the absence of family history, the cause may remain unknown despite comprehensive investigations. This group, categorised with the descriptive term “idiopathic cerebellar ataxia” [2] could be considered as consisting of cases with heterogeneous ataxic syndromes, including Multiple System Atrophy (MSA), immune-mediated or other acquired ataxias, and undefined inherited ataxias [3, 4]. It is suspected that many of the idiopathic CA cases are genetic and, with the advances in genetic testing, this third group is becoming a decreasing proportion of all cases [5].

CA can present at any age and are traditionally classified as early (<20 years) or late onset [6]. Genetic CA can follow an autosomal dominant (AD), autosomal recessive (AR), X-linked, or mitochondrial mode of inheritance [7].

The hereditary CA are clinically and genetically heterogeneous neurodegenerative disorders characterised in pure forms by a cerebellar syndrome with a slowly progressive

combination of gait instability, limb incoordination, dysarthria and eye movement abnormalities [8]. CA may manifest as more complex syndrome, characterised by the presence of additional neurological features, including pyramidal signs, cognitive dysfunction or peripheral neuropathy [9] and a variety of non-neurological features [7, 10].

A diverse range of ophthalmic abnormalities have been reported in patients with ataxias and other inherited neurodegenerative diseases [11] and in some patients, ophthalmic manifestations are the earliest symptoms of inherited neurodegenerative disease.

1.2 Prevalence of inherited ataxias

The global prevalence values for CA across the world are highly variable. The prevalence range of ARCA collectively, including Friedreich's ataxia (FRDA) and non-FRDA is reported at 0.0-7.2:100 000 [12]. FRDA is recognised as the most prevalent hereditary ataxia in the Caucasian population [5, 13] with an estimated prevalence in Western Europe between 1:20 000 and 1:50 000 and estimated carrier frequency ranging from 1:60 - 1:110 [14, 15]. Ireland has been reported to have one of the highest observed FRDA prevalence at 1:23 000 [15]. Spastic paraplegia 7 (*SPG7*) is the second most common ARCA worldwide [5, 16], followed by Ataxia telangiectasia (A-T) [13, 17] and Ataxia with oculomotor apraxia (AOA), found to be more common in Portugal and Alsace [18, 19], with AOA1 reported as the most frequent ARCA in Japan [13, 20]. Based on allele frequency the estimated disease prevalence for CANVAS, a common cause of adult onset ataxia ranges from 1:10 000 to 1:650 individuals.

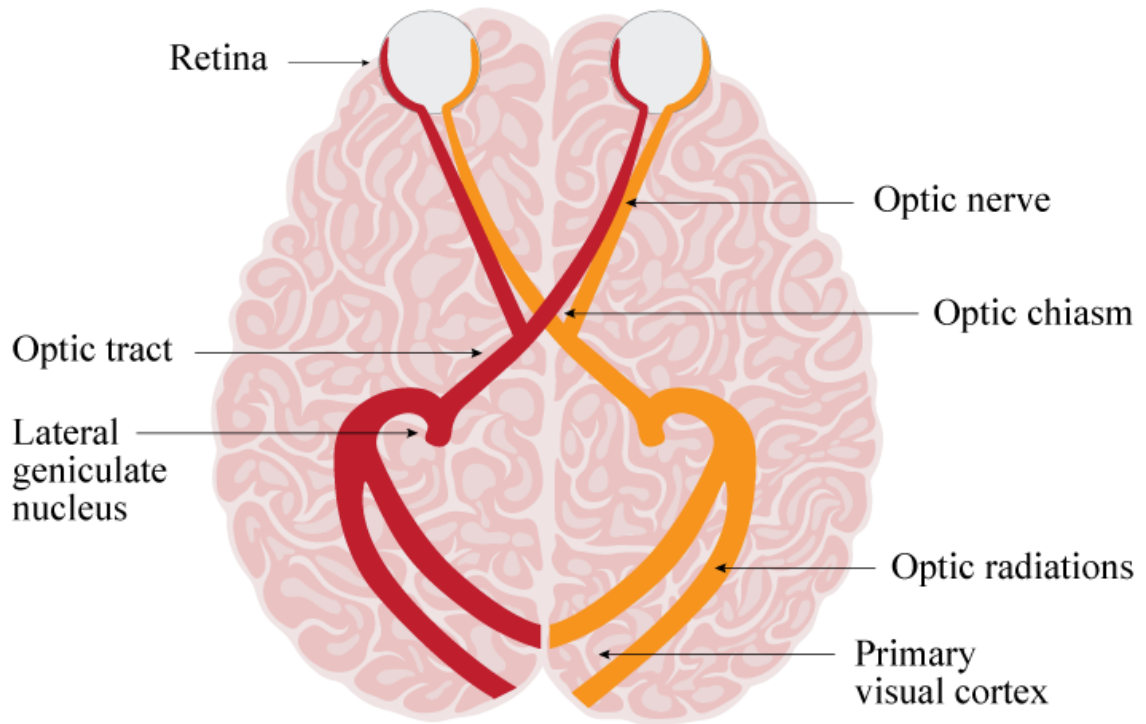
Global prevalence of ADCA ranges between 1.5–5.6:100 000 [12]. The most frequent forms are caused by polyglutamine (polyQ)-coding CAG trinucleotide repeat expansions in seven genes: *ATXN1* (SCA1), *ATXN2* (SCA2), *ATXN3* (SCA3/Machado-Joseph disease), *CACNA1A* (SCA6), *ATXN7* (SCA7), *TBP* (SCA17), *ATN1* (dentatorubral-pallidoluysian atrophy) [21, 22]. SCA3 is the most common ADCA worldwide with the highest relative frequency in Brazil [23], followed by SCA2, the commonest subtype in Cuba, and SCA6 [12, 24]. Despite comprehensive investigations, systematic approach in evaluation of individuals with CA, and recent advances in genetics, a substantial proportion of individuals still remain genetically undiagnosed [5, 25].

1.3 Efferent and afferent visual systems

The visual system is composed of the afferent and efferent visual pathways. The latter account for the ocular motor part of vision and facilitate eye movements that allow for an in-focus view of objects to capture visual information. A wide range of eye movement abnormalities (abnormal smooth pursuits, saccadic deficits, etc) are among the most common phenotypic manifestations of patients with neurodegenerative diseases [26].

The afferent visual pathway involves all the structures responsible for receiving, transmitting, and ultimately processing visual information [27]. These include the eye, optic nerves, chiasm, tracts, lateral geniculate nucleus (LGN) of the thalamus, radiations, striate cortex, and extra striate association cortices (Figure 1.1). Visual information is initially captured by light-sensitive photoreceptors in the retina and directed through various synaptic connections to reach the retinal ganglion cells (RGCs) in the inner retina. The axons of the RGCs form the optic nerve and the two optic nerves meet at the optic chiasm, where the nerve fibres originating in the nasal retina of each eye cross to join the temporal fibres of the fellow eye. From the chiasm, the same axons continue on as the optic tract and travel to and synapse on the LGN of the thalamus. From the LGN, the optic radiations carry information to the primary visual cortex in the occipital lobe for initial visual processing and then to various extra striate cortices for higher-level processing including visual recognition and visuospatial processing [27, 28].

Figure 1.1 Afferent visual system



Visual information, captured by light-sensitive photoreceptors in the retina is transmitted through the optic nerve and optic tract, which directly synapses on the LGN of the thalamus. From the LGN, the information is carried through the optic radiations to the primary visual cortex in the occipital lobe for initial visual processing.

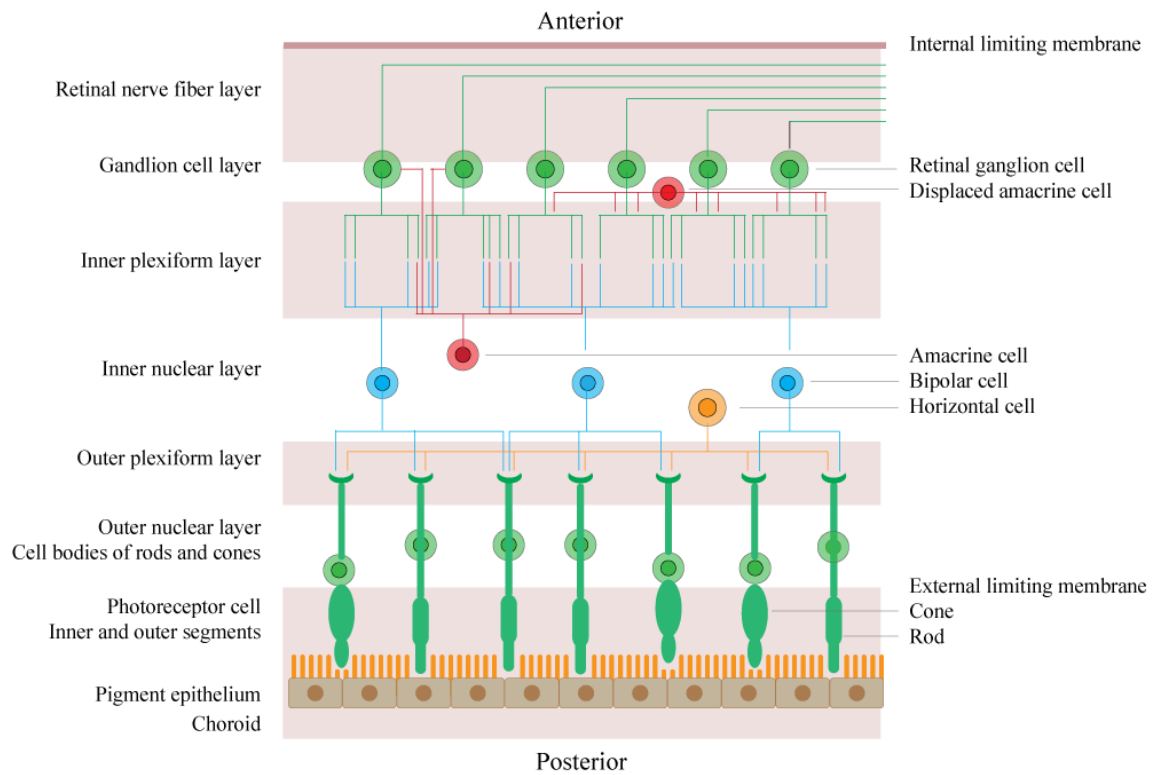
Adapted from [28]

1.4 The retina

The retina is a light-sensitive tissue that lines the back of the eye, upon which the images of external objects are received. The retina is soft, semitransparent, and of a purple tint in the fresh state, owing to the presence of the colouring material rhodopsin, which becomes clouded, opaque, and bleached when exposed to sunlight [29]. In the centre of the posterior part of the retina, corresponding to the axis of the eye, is the macula, which is responsible for central vision. At the fovea, seen as a small depression in the centre of macula, visual acuity is the highest. About 3 mm to the nasal side of the macula is the optic disc, the entrance of the optic nerve with the retinal artery piercing the centre of the disc.

The retina is a multi-layered structure, consisting of six different types of cells divided into ten distinct layers of neurons, interconnected by synapses (Figure 1.2). The six different cell types in the retina include rods, cones, unmyelinated RGCs, bipolar cells, horizontal cells and amacrine cells. Each of these neurons plays a specific role in creating and transmitting vision [30]. The axons of about 1.2 million RGCs within the inner retina constitute the retinal nerve fibre layer (RNFL) and converge to form the optic nerve [31]. These axons leave the eye at the optic disc and act as the most proximal part of the afferent visual system. They represent therefore a unique part of the central nervous system, as the retina is the only extension of the brain that can be viewed. This is of significant importance as changes in the retina, affecting the RNFL, primarily represent axonal loss [32].

Figure 1.2 Schematic illustration of retinal structure



Adapted from [33]

1.5 Ophthalmic manifestations in inherited ataxias

Ophthalmic findings occur frequently in neurodegenerative disorders. A diverse range of ophthalmic abnormalities, especially affecting the optic nerve and retina, as well as eye movements, have been reported in patients with CA. Clinically, visual abnormalities can be a clue to diagnosis and may precede other symptoms in certain types of CA, such as SCA7, besides being a prominent cause of disability in affected individuals [11].

The entirety of the visual system can be involved, for example both afferent and efferent visual abnormalities have been described in FRDA [34]. Visual disturbances in ADCA mainly reflect efferent visual system problems and while no individual sign is pathognomonic, some ophthalmologic signs are far more prevalent in specific SCAs (Spinocerebellar ataxias), for example downbeat nystagmus in SCA6, proptosis in SCA3 or prominent slow saccades early in the disease course in SCA2 [35 - 37]. In addition, abnormal afferent visual testing and optic atrophy have been described as part of the phenotype in some of the SCAs [38] with retinal pathology being a particularly prominent feature in SCA7 [39]. Previous research suggests that optic nerve involvement is more common in the neurodegenerative disorders in which mitochondrial dysfunction has a central role, such as FRDA and some hereditary spastic paraplegias (HSPs) [40].

1.6 Optical coherence tomography

Optical coherence tomography (OCT), a light interference based optical technique, allows three-dimensional cross-sectional imaging within biological tissues with a high resolution. OCT is a non-invasive and powerful imaging technology which can function as a type of optical biopsy and, unlike conventional histopathology which requires removal of a tissue specimen and processing for microscopic examination, OCT provides *in vivo* images. Furthermore, fast scanning rates and quick signal processing allow for image visualisation in real time [41].

1.6.1 Historical background

An early use of optical interferometry in the biomedical field was first reported by Simonsohn et al. in 1967 [42], who determined the refractive index distribution of the animal eye lenses. A decade later, laser interference fringes, projected on the fundus of the human eye, were used by Rassow et al. for *in vivo* retinal resolving power measurement [43]. Initial experiments in ophthalmologic length measurement interferometry in the early 1980s suggested that laser interferometry can be used for *in vivo* distance measurement on human eyes [44]. The first retinal imaging was performed in a laboratory in 1989 and, two years later, Huang et al. demonstrated the earliest OCT scans of a human retina and coronary artery *ex vivo* [45]. Moreover, they were able to compare OCT images with histology of the same tissue and describe the structures that could be seen using this first-generation prototype time-domain OCT (TD-OCT). The first OCT cross-sectional images of biological microstructure were produced in 1991, before Fercher et al. presented, first *in vivo*, ocular imaging in 1993, and subsequently showed the first retinal disease images in 1995 [46]. A decade later, a considerable step

in the evolution of OCT was made with the introduction of light wave lengths instead of time delay to determine the spatial location of reflected light. Based on the original method of TD-OCT, advancements in technology led to the development of spectral-domain OCT (SD-OCT), also known as Fourier domain OCT [47]. The noteworthy advantages of SD-OCT include ultra-fast frequency scanning light source, faster scanning speed, higher sensitivity, and superior high resolution, especially for more accurate segmentation of the RNFL.

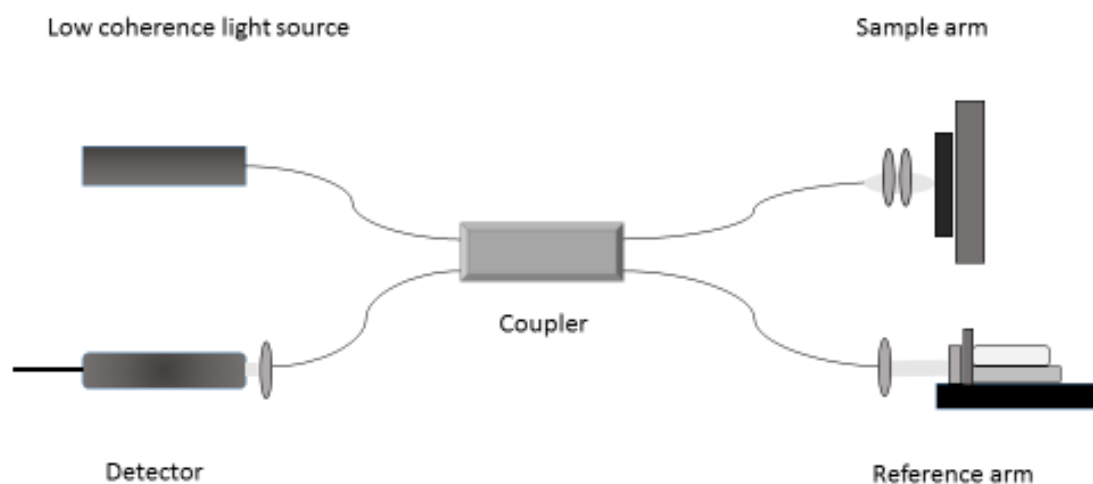
Since the commercial OCT became available in 2002, this technology continues to evolve and from an optical imaging method used mostly in research laboratories in the late 1990s, has become a valuable tool used in ophthalmology and is currently considered the “gold standard” for retinal imaging. Furthermore, significant technological improvements in the last two decades have allowed the widespread application of OCT in a variety of medical fields, such as interventional cardiology, dentistry, gastrointestinal endoscopy, surgical guidance, dermatology, laryngology, gynaecology, respiratory tract diseases and neurology.

1.6.2 Principles of optical coherence tomography

OCT is often considered an optic analogue of B-mode ultrasonography [32]. Both imaging techniques direct waves to the examined tissue and the waves echo off the tissue structure. The back reflected waves are analysed and their delay is measured to reveal the depth in which the reflection occurred. OCT uses light in the near-infrared, which travels much faster than ultrasound. The delays of the back reflected waves cannot be measured directly, thus a reference measurement is applied. Through the use of an interferometer, part of the light is directed to the sample and another portion is sent to a reference arm with a well-known length.

The functional principle behind OCT imaging is light interference. The basic concept of light interference using the optical fibre-based Michelson setup is shown in Figure 1.3. In an OCT system, the light from a low-coherence source is split into two paths by a coupler, which is directing it along two different arms of an interferometer. One arm is designated as the reference arm, while the other is the sample arm. When the light exits the fibre end of either arm, it is shaped by various optical components (mirrors, lenses, etc.) to control specific beam parameters such as shape, depth of focus, and the intensity distribution of the light. In the reference arm, the light is back-reflected by a reference mirror and it returns into the interference system, propagating along the same path it came from but in the opposite direction. The same process happens with the light in the sample arm with the only difference being that the beam is backscattered by the sample.

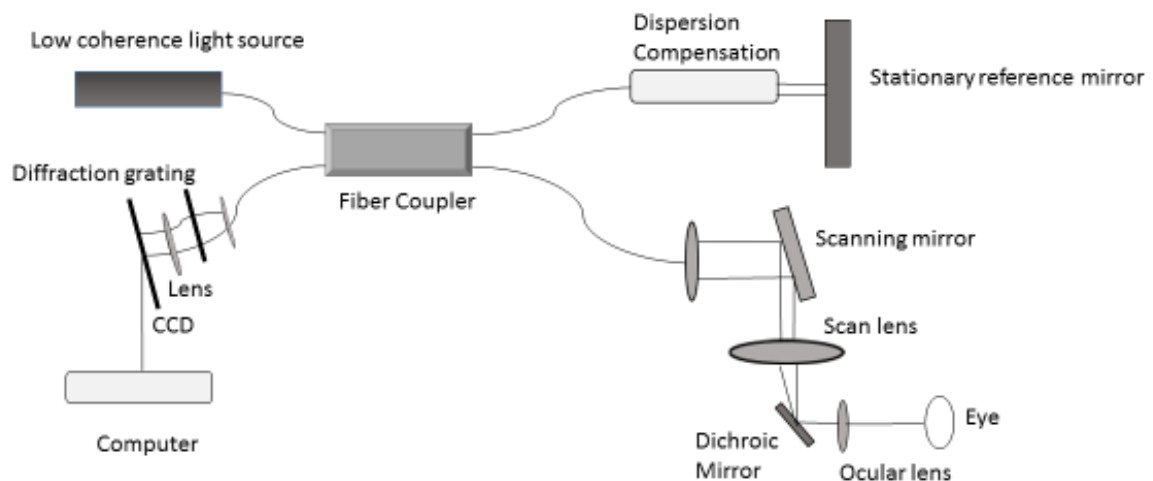
Figure 1.3 Fibre-based OCT system in a Michelson configuration



The light from a low-coherence source is split in two by the coupler with each part traveling along a separate arm of the interferometer, the reference and the sample arm. The light backscattered from the reference mirror and from the sample recombine at the coupler and generate an interference pattern, which is recorded by a single point detector. Adapted from [48]

In contrast to TD-OCT where the reference mirror is moving, the mirror is stationary in SD-OCT (Figure 1.4). The interference pattern is split by a grating into its frequency components and all of these components are simultaneously detected by a charge coupled-device (CCD). The CCD has a number of photodetectors each sensitive to a range of specific frequencies.

Figure 1.4 Schematic illustration of spectral-domain optical coherence tomography



In SD-OCT the reference mirror is stationary. Adapted from [49]

Standard OCT systems use time-domain detection, achieving scan rates of 400 A-scans per second and an axial resolution of 8-10 μm [50]. From the technological perspective, the advances in SD-OCT have a huge impact, providing higher sensitivity, much higher speed of data acquisition (greater than 20,000 A-scans per second) and better resolution (5-7 μm), thus allowing for *in vivo* acquisition of large volumetric data sets in a shorter time frame [50 - 52].

1.6.3 Retinal imaging in neurological diseases

A direct bedside visualisation of the retina and optic nerve was first achieved in 1851 following the introduction of the hand-held ophthalmoscope by Helmholtz [53]. Over a century later, Frisén and Hoyt [54] reported their subjective analysis of thinning of the RNFL, principally composed of axons from ganglion cell neurons, as evaluated with hand-held ophthalmoscopy, in patients with Multiple Sclerosis (MS). Their observations corroborated in a postmortem study that demonstrated atrophy of the RNFL (typically containing about 80% axons and 20% glia) in 35 / 49 eyes evaluated from patients with MS [55]. However, this study was not sufficiently quantitative to enable a full appreciation of the relationship between vision, RNFL thickness, and the integrity of the optic nerve.

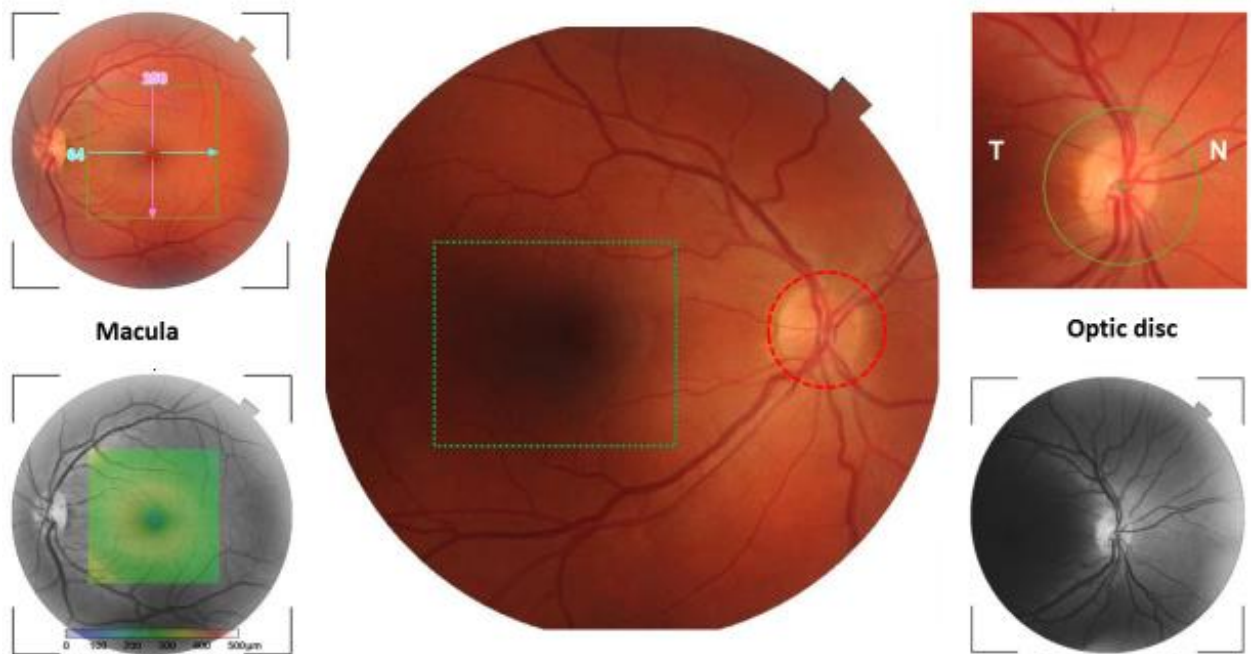
Subsequent development of OCT technologies has allowed for improved visualisation of the retina, which in the past decade has become an essential part of assessment since visual function has been shown to be directly related to the integrity of retinal anatomy.

The ocular structures of interest for evaluation with OCT in neurological diseases are the macula and the optic nerve (Figure 1.5). As already mentioned, OCT allows imaging of the RNFL, a structure that contains ganglion cell axons which form the optic nerves [32]. Since these axons are non-myelinated within the retina, the RNFL is an ideal structure with which to visualise the processes of neurodegeneration, neuroprotection and, potentially, even neuro-repair. In contrast to the peripapillary RNFL, which contains axons, the macula contains a large proportion of retinal ganglion cell neurons (about 34% of total macular volume) [56]. About 1200 μm in diameter, the macula is easily identified within the retina.

The application of non-conventional magnetic resonance imaging (MRI) techniques, such as magnetisation transfer imaging, magnetic resonance spectroscopy, and diffusion tensor imaging, has led to only modest achievements in linking imaging data with the clinical measures of disease severity for neurologic disorders. On the other hand, OCT has been increasingly incorporated as an exploratory outcome in treatment trials, observational studies, and even in clinical practice in order to achieve a greater understanding of the relationship between changes in retinal structure and patient reported outcomes of visual function [57].

The macular images obtained by OCT demonstrate the architecture of the various retinal layers at the cellular level. Moreover, the OCT can directly measure and quantify the optic nerve head [58].

Figure 1.5 Specific retinal zones that are imaged when assessing the retinal nerve fibre layer and macular thickness



Retinal fundus image of a healthy subject obtained with Topcon SD-OCT.

Scanned areas: Macula (Left) and Optic disc (Right) with colour photo (top) and red-free photo (bottom) shown.

1.6.4 Factors affecting OCT imaging

1.6.4.1 Subject related factors

Although one study [59] did not show significant association between RNFL thickness and age, a number of subsequent studies have reported that RNFL thickness decreases with age [60, 61]. This finding is consistent with histological studies, which have shown age-related decline in axon numbers in the optic nerve [62].

Variability in the RNFL thickness among different ethnic groups has been documented. Budenz et al. [60] reported significantly lower mean RNFL thickness in Caucasians than in subjects of Hispanic or Asian origin, while Kelty et al. [63] documented significantly smaller mean foveal thickness in African-American individuals compared with Caucasians.

The relationship between the RNFL thickness and myopia has been extensively investigated and is still debated. Hoh et al. [64] reported no correlation, while Budenz et al., and Leung et al. [60, 65] observed that the average RNFL thickness decreased with myopia. Lim and Chun [66] compared the peripapillary RNFL thickness of high myopic eyes ($SE \leq -6.0$ D) with those of low myopic eyes (SE from -0.25 to -3.0 D) in children and found that the mean overall thickness of the peripapillary RNFL in the high myopic subjects was significantly lower than that in the low myopic subjects. Another study [67] reported that the mean peripapillary RNFL thickness was thinner in highly and moderately myopic eyes compared with low myopic eyes.

Any potential effect of myopia may not be uniform. Some studies have reported that with myopia and an increase in the axial length, the regional thickness of the superior and inferior peripapillary RNFL decreases. In 2016, Choi et al. [68] reported slight and statistically insignificant increase in the thickness of the temporal RNFL in myopic eyes, while Kang et al., and Zha et al. [69, 70] observed that the high myopia group had significantly reduced RNFL thickness in the non-temporal sectors, but significantly increased RNFL thickness in the temporal quadrant in comparison to low-to-median and emmetropic (control) groups. Overall, these findings suggest that myopic eyes (especially in cases of high myopia) have a thicker temporal RNFL.

1.6.4.2 Analytical factors

Numerous studies have demonstrated good reproducibility and repeatability of SD-OCT to measure RNFL and macular thicknesses both in normal eyes and those with disease [65, 71]. However, despite significant hardware and software developments in OCT, SD-OCT imaging artefacts are a common finding in clinical practice that can result in poor scan quality [72].

Measurement of the OCT can be influenced by artefacts arising as a result of either operator error or data-processing software features (Table 1.1).

Table 1.1 Most common factors affecting the OCT scan quality

Patient – related
Pupil size
Patient cooperation
Eye pathology (cataracts, vitreous opacities, poor BCVA, drusen, high myopia, dry eye)
Blinking artefacts
Movement artefacts
Procedure-related (operator-dependent and device-dependent)
Scan alignment
Scan centration
Signal strength variability
Opacities of the OCT lens

1.6.5 Nomenclature when using OCT

Consensus nomenclature for normal anatomic landmarks on spectral-domain optical coherence tomography (SD-OCT) images of a normal eye was adopted in 2014 by the International Nomenclature OCT (INOCT) Panel [73].

The Advised Protocol for OCT Study Terminology and Elements recommendations, which include core items to standardise and improve quality of reporting in quantitative OCT studies was developed by consensus [74]. The areas covered include: study protocol, acquisition device, acquisition settings, scanning protocol, funduscopy imaging, post-acquisition data selection and analysis, recommended nomenclature, and statistical analysis (Appendix 1).

1.7 Applications of OCT in clinical practice

Over the past decade, OCT has transformed ophthalmic clinical practice, becoming one of the most important tests in ophthalmology. The widespread use of OCT is clinically relevant for the diagnosis and management of a variety of conditions, including glaucoma, age-related macular degeneration, diabetic retinopathy, as well as intraocular tumors, inherited syndromic and non-syndromic retinal diseases. OCT is also used in ophthalmological surgery [75].

In the last number of years, OCT has expanded from a widely used diagnostic tool in ophthalmology into neurology. OCT has been applied in several areas, demonstrating its potential role in the study of neurodegenerative diseases [76]. RNFL thinning has been documented in patients with MS and Neuromyelitis optica (NMO) [32, 77, 78]. The potential application of retinal evaluation using OCT to provide better understanding of the possible role of RNFL as a biomarker for the detection of neurodegeneration in Alzheimer's disease, Parkinson disease (PD) and MSA-C has also been explored [38, 79, 80]. Moreover, cross-sectional and longitudinal OCT studies of patients with MSA-C have reported significant and progressive RNFL thinning even in visually asymptomatic individuals [81].

The role of OCT has also been demonstrated in other neurological diseases, including epilepsy to detect drug-induced retinopathy in patients treated with vigabatrin [82]. OCT is increasingly used for assessment of fingolimod-associated macular oedema which has been documented in patients with MS, typically occurring within the first 3–4 months after initiation of treatment [83]. Furthermore, this technique has become a valuable tool in the care of patients with papilloedema and idiopathic intracranial hypertension [84]. In recent years, a number of studies have investigated RNFL thickness changes in patients with migraine compared with healthy subjects [85],

expanding the utility of this non-invasive, non-expensive and increasingly available technology.

More recently, intravascular OCT has been explored for use in the neurovascular field for guiding endovascular treatment of ischaemic stroke and brain aneurysms [86]. OCT has shown its potential for the imaging of neurovascular disease [87] and an intravascular OCT imaging catheter design tailored for use in tortuous neurovascular anatomy was proposed in 2020 [88]. Outside ophthalmology and neurology, OCT has been used in interventional cardiology, dentistry, dermatology, gastrointestinal oncology with endoscopic OCT, gynaecology and laryngology among others [89, 90].

1.8 Optical coherence tomography in inherited ataxias

Various studies have demonstrated that abnormal retinal thickness may occur in patients with inherited ataxia syndromes to a degree measurable on OCT [38, 91].

1.8.1 Friedreich's ataxia

FRDA is the most frequent autosomal recessive cerebellar ataxia, caused by defect in the frataxin (*FXN*) gene on chromosome 9q13-q21.1,57 [92]. The vast majority of individuals with FRDA are homozygous for a GAA triplet repeat expansion in the first intron while a small proportion of patients are compound heterozygous for a repeat expansion and carry a point mutation on the other allele [92]. The nuclear-encoded mitochondrial protein frataxin is directed to the mitochondrial inner membrane and is involved in iron-sulfur protein homeostasis within mitochondria. Genetic abnormalities in *FXN* gene lead to decreased level of frataxin, resulting in impaired oxidative phosphorylation, decreased bioenergetic output, increased reactive oxygen species, iron accumulation in mitochondria, and apoptosis [93, 94].

Until relatively recently, ocular motor abnormalities were the best-characterised signs of visual system damage in patients with FRDA, reflecting disruption of brainstem-cerebellar circuits [95]. However, afferent visual pathway involvement, including optic neuropathy, has also been described in up to two-thirds of affected individuals [96]. With advances in ocular imaging techniques, involvement of the afferent visual pathways in FRDA has been further investigated, with particular emphasis on OCT findings [34, 96 - 98]. These studies have described the RNFL changes associated with FRDA and correlations with disease severity and disease duration. In 2018, Parkinson et al. reported that FRDA was associated with the greatest degree of RNFL thinning in comparison to a range of other genetically characterised ataxias [91].

Fortuna et al. (26 patients), Dağ et al. (10 patients / 10 eyes) and Seyer et al. (57 patients / 110 eyes) have reported statistically significant diffuse / average RNFL thickness reduction in comparison to controls. Decreased average peripapillary RNFL thickness was also reported by Noval et al. (23 patients) and a distinctive pattern of RNFL loss predominantly in the superior quadrant was documented in one of the studies [34].

Macular thickness in FRDA was normal in one study [97], but below the first percentile for age-matched controls in another, where 29 eyes were evaluated [34], and significantly reduced foveal thickness was documented by Dağ et al. [98].

RNFL thickness has been shown to correlate with neurological function and disability as measured with the International Cooperative Ataxia Rating Scale (ICARS) [96 -97], although not consistently with Friedreich's Ataxia Rating Scale (FARS) [34, 97]. To date, two studies have evaluated the correlation between the peripapillary RNFL and the Scale for the Assessment and Rating of Ataxia (SARA) used to quantify disability [99] (Appendix 3) in FRDA patients [100, 101]. Both the larger UK (52 patients) and the small Spanish (8 patients) studies found that SARA score was correlated with RNFL thickness, demonstrating that as cerebellar function declines, the RNFL becomes thinner. Furthermore, a significant correlation between ADLQ (Activities of Daily Living Questionnaire) score from the modified FARS assessment and RNFL thickness was documented in the UK cohort.

The relationship between the RNFL thickness and the age of onset has been documented in several studies [34, 96, 101], while correlation between RNFL and disease duration, reported by Noval et al., Seyer et al., and Thomas-Black et al., was not seen by Fortuna et al. No relationship between RNFL and age onset and disease duration was documented in the small Turkish study [98].

Altogether these findings indicate that RNFL structural changes have a functional correlate, thus suggesting that OCT measurements, and in particular RNFL parameters, are a useful marker of disease progression in FRDA patients.

1.8.2 Spinocerebellar ataxias

The Spinocerebellar Ataxias (SCAs) are a heterogeneous group of ADCA and more than 40 genetically distinct subtypes have been described. SCAs are designated by a number indicating the chronological order in which the disease locus was first identified; the most recently described subtype is SCA48 [102]. Pathogenetic classification divides SCAs into two main groups: polyglutamine (polyQ) SCAs, caused by CAG repeat expansions resulting in abnormally long chains of glutamine residues in the encoded proteins, and SCAs caused by standard mutations (non-repeat expansion). The most common SCAs worldwide are SCA1, 2, 3, 6 and 7, representing approximately 80% of all ADCA [21, 22].

Previous reports demonstrated that ophthalmic features present in affected individuals can aid distinction of different SCA types. The efferent system is most commonly affected, but subclinical or clinical involvement of the afferent visual system has also been observed [103], with optic nerve dysfunction reported in patients with different types of SCA.

OCT has been applied to study retinal changes in the most common trinucleotide expansion SCAs (SCA1, SCA3, SCA7) and also in mixed SCA cohorts [104]. A study of nine patients with SCA1 reported statistically significant average peripapillary RNFL loss compared to healthy controls with most prominent reduction in the temporal region [105]. However, these findings were not replicated in another study of seven SCA1

patients [38]. In contrast to the latter, average peripapillary RNFL thickness was reduced in patients when compared to controls in a larger study of 20 SCA1 individuals [106].

Macular dysfunction in association with SCA1 has also been documented, initially in a small number of patients [107, 108]. In a more recent study, 25% (5/20) of patients with SCA1 displayed a distinct maculopathy, characterised by a disruption of the ellipsoid zone (EZ disruption), suggesting retinal involvement in this entity in addition to widespread neurodegeneration [106].

Stricker et al. [105] did not find correlation between RNFL thickness with disease duration, disease severity as measured with SARA score, and visual acuity in SCA1 patients, while Oertel et al. reported no association between RNFL thickness with disease onset and severity as quantified with SARA [106].

Data on OCT findings in patients with SCA2 and SCA3 are limited. Mild reduction in RNFL thickness as detected by OCT has been reported in 9 patients with SCA3. In 15 of 18 eyes the average RNFL thickness was lower than the population average [109]. Temporal sector thickness was preserved in all, but reduction in superior, inferior and nasal sectors has been identified in some eyes. A negative correlation was found between RNFL thickness and SARA score, but there was no significant relationship between RNFL measures and disease duration.

In a study of patients with SCA1 (7 patients), SCA2 (7 patients), SCA3 (5 patients) and SCA6 (5 patients) [38] subjected to OCT, average RNFL thinning was observed in SCA2 and SCA3 groups only, when compared to normal controls. Furthermore, this study reported that the overall thickness in the macular region was significantly thinner in SCA1, SCA3 and SCA6, but not in SCA2 subgroups. Disease severity as quantified

with SARA score was inversely correlated with the OCT measurements in the SCA2 and SCA3 subgroups.

A comparative OCT study of SCA3 (10 patients) and SCA10 (9 patients) [110] showed that RNFL had a tendency to be thicker in patients with SCA10 than in those with SCA3, but did not reach statistical significance. Changes in RNFL were observed in at least one region in 6/10 individuals with SCA3 and in only 2/9 SCA10 patients. Retinal changes in affected SCA3 cases demonstrated significant nasal RNFL thinning, which was inversely proportional to SARA score used to quantify disease severity. The results from this study suggested that in SCA10 RNFL thickness changes are less prominent and unrelated to disease duration.

Disease-specific involvement of the afferent visual system has been demonstrated in some SCAs, but is most prominent and well described in SCA7 [111]. Visual loss, blue–yellow colour vision defects and blurred vision may occur years before other manifestations of the disease. The central vision becomes affected first and can progress to complete blindness. Retinal changes might be very subtle initially, affecting the central macula, and subsequently advance to cone-rod dystrophy, the underlying cause of progressive vision loss in SCA7 [112].

Retinal thinning with loss of the peripapillary RNFL has been demonstrated by OCT in SCA7 patients with gradual loss of vision [113], with the retinal thinning extending outside the visibly atrophic lesions [114].

A recent study of 16 patients with SCA7 documented various degree of maculopathy with foveal atrophy present in all 9 individuals who had topographic macular analysis, while the average RNFL thickness was normal in majority of individuals [115]. No

correlation was sought between retinal changes and disease severity, but an inverse association was found between SARA score and endothelial corneal cell density.

Similar findings were also documented in another study [116]. Foveal thinning on OCT was observed in all symptomatic SCA7 cases (13 patients) but not in controls (5 individuals) and pre-symptomatic carriers (3 individuals). Interestingly, in this study visual fields and Spinocerebellar Ataxia Functional Index (SCAFI, Appendix 2) [117] were significantly correlated with time to disease onset (pre-symptomatic) / disease duration (symptomatic carriers); SCAFI showed a trend to differentiate pre-symptomatic carriers from controls.

Structural changes in the retina and optic disc as detected with an OCT have been rarely reported in other types of SCAs. SCA14 (SCA-PRKCG) was one of the first SCAs in which a conventional mutation (in the *PRKCG* gene) was described [118]. The protein kinase C gamma, expressed in neurons of the brain and the spinal cord, is also found in the visual system, especially in the retina [119]. A study of 12 individuals with SCA14 showed that although visual acuity and contrast sensitivity, as well as reported vision-related quality of life were worse in patients than in age-matched controls, there were no significant changes in RNFL and macular thickness in the patient group [120]. In addition, no association was observed between RNFL thickness and disability as quantified with SARA score. The results from this study suggested, that retinal thinning is neither a prevalent, nor disease specific finding in SCA14 and therefore, retinal atrophy does not contribute to visual impairment in this cohort.

Retinal examination of patients with SCA, especially in those subtypes known to be associated with ophthalmic involvement, is therefore of potential value in both clinical and research settings. OCT has been shown to have potential as a marker of disease

progression in some SCAs, although given small sample sizes may be more relevant for group analysis rather than for individuals.

1.8.3 Autosomal recessive spastic ataxia of Charlevoix-Saguenay

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a rare neurodegenerative disorder, first described in individuals from Quebec. ARSACS typically presents as an early-onset cerebellar ataxia, slowly progressive lower limb spasticity and axonal-demyelinating sensorimotor peripheral neuropathy [121]. ARSACS is caused by mutations in the *SACS* gene located on chromosome 13q12.12 [122]. The *SACS* gene encodes saccin, a protein highly expressed in cerebellar Purkinje cells, brainstem nuclei, and in the large pyramidal forebrain neurones [123], thought to have a role in the regulation of mitochondrial dynamics, leading to mitochondrial dysfunction [124].

Abnormal thickening of RNFL is the characteristic retinal change typically visible on fundoscopy in patients with ARSACS [125]. However these retinal changes are not consistently observed in non-Québécois cases.

In the last decade, OCT has shown to be a beneficial tool in detecting RNFL changes in ARSACS. Following initial small studies showing marked global thickening of the peripapillary RNFL with loss of the foveal depression in individuals with ARSACS [126, 127], Parkinson et al. characterised the OCT findings in a cohort of 17 patients with ARSACS and 13 asymptomatic heterozygous ARSACS carriers [91]. Although only 70% of affected individuals in the study had peripapillary retinal striations visible on fundoscopy, all had abnormal thickening of the RNFL on OCT which was not observed in the evaluated controls and subjects with other genetically confirmed ataxias. Peripapillary RNFL thickening was also documented in all 13 affected ARSACS

Brazilian patients in Filho et al. study [128]. These studies found that abnormal RNFL thickening appears to be a sensitive and specific biomarker of ARSACS disease, thus routine use of OCT technique in the assessment of all suspected cases, even in the absence of fundusoscopic changes, has been proposed.

1.8.4 Hereditary spastic paraplegias

Hereditary spastic paraplegias (HSP) are clinically and genetically diverse group of disorders [6], in which the primary symptom is progressive walking difficulty due to lower limb spasticity and weakness. The HSP are classified clinically as pure forms when symptoms are limited to progressive spastic weakness in the legs, and complex (complicated) HSP forms if additional neurological, and non-neurological signs are present. In the last number of years the discovery of more and more genes causing both predominantly pyramidal and prominent cerebellar phenotypes has demonstrated the significant overlap between CA and spastic paraplegia [129]. Cerebellar and pyramidal presentations commonly occur together and can vary considerably in predominance and phenotypic expression along the disease spectrum.

Small case series have reported subclinical optic neuropathy even in HSP patients with little or no evidence of visual dysfunction, detected as an abnormal thinning of the RNFL to a degree measurable by OCT. To date, a handful of studies have investigated the OCT findings in individuals with HSP to evaluate the pathology of HSP and see if RNFL thinning, seen in other neurodegenerative disorders, can be found in this group of disorders, characterised by distal axonopathy [130, 131].

An OCT study of 28 HSP patients, clinically divided into pure (n=22) and complex forms (n=6), included cases with *SPG4* (13), *SPG5* (1) and *SPG7* (3), while 11

individuals were genetically undetermined. Significant reduction was found in temporal and temporal inferior quadrants on OCT of patients with complex but not with pure phenotypes. No correlation was found between global RNFL thickness and age of onset, disease duration and disease severity as quantified with the Spastic Paraplegia Rating Scale (SPRS). Another study of 134 individuals with HSP, included 10 individuals with *SPG7* and reported evidence of RNFL thinning as detected by OCT [131].

More recent OCT study reported a cohort of 23 genetically highly heterogeneous HSP patients [132]. Mostly mild-to-moderate thinning of the RNFL was documented, which was not a constant feature of complex HSP, but was more widespread and extended to the pure forms. RNFL changes did not affect any specific quadrants, but notably spared the temporal area. However, the sample size with only 2 *SPG7* patients included, was very small for meaningful conclusions for this entity. In this study, observed RNFL thinning correlated with age and disease duration, but not with clinical severity as quantified with the SPRS.

Mutations in the *C12orf65* gene, involved in the process of mitochondrial translation, have been linked to a spectrum of phenotypes, including early onset optic atrophy, progressive encephalomyopathy, peripheral neuropathy, and spastic paraparesis, classified as the rare AR *SPG55* [133]. A single previous report documented general reduction in the RNFL thickness as detected with OCT in affected individuals [134].

OCT in patients with Kjellin syndrome, a neuroophthalmologic presentation linked to mutations in *SPG11* and *SPG15*, initially identified in association with pigmentary maculopathy, and subsequently with an increased risk of developing psychosis [135], showed that retinal changes are only observed once the paraplegia becomes apparent [136].

Optic atrophy has also been described in patients with *SPG35* [137], but no studies have documented OCT findings.

1.8.5 Prospectively assessed retinal changes in inherited ataxias

There is limited data on the utility of OCT as a tool in assessing RNFL changes over time and its potential to serve as a biomarker for disease progression.

Rojas et al. documented macular and RNFL thickness decline over 6 months period of time in 8 prospectively evaluated with OCT FRDA patients [100].

In four out of nine patients with SCA3 OCT was performed during a mean follow-up of 14 months and showed mild trend to RNFL thickness decrease in 62.5% [109], suggesting mild and slowly progressive RNFL thinning.

A comprehensive study of pre-clinical and clinical phases of SCA7 has demonstrated progressive deterioration of macular thickness as seen on OCT, as well as gradual worsening of SCAFI and visual fields, suggesting that these tools can be considered interesting candidates for state biomarkers for SCA7 since pre-symptomatic stages [116].

Nine of twenty three individuals of a highly genetically heterogeneous HSP cohort [132] had a follow-up OCT assessment (mean 10.7 months). No significant change in OCT measurements compared to the baseline data was found.

A small study of affected individuals with *C12orf65* – associated HSP did not show any further reduction in the RNFL in the three individuals followed up after 4 years [134], indicating a very slow progression of the optic neuropathy.

This study will prospectively evaluate a large cohort of patients with progressive ataxia attending the only designated centre of expertise for the investigation and management of CA in the Republic of Ireland. This research will focus on investigation of retinal changes associated with different subtypes of CA using OCT, and thus provide information on the observed RNFL thickness pattern, describe correlations with measurements of disease severity and disease duration, and document if RNFL changes are evident over time. This study will further explore if OCT, a non-invasive, highly reproducible and relatively easy to use technique, has the potential to serve as a sensitive tool for diagnosis and monitoring of disease progression in different subtypes of inherited ataxia.

Chapter 2: Hypothesis and aims

2.1 Main hypothesis

Optic nerve atrophy, recognised as a disc pallor on fundus examination, is well described in individuals with various types of inherited ataxias. Despite clinical and genetic heterogeneity a number of reports suggest that retinal nerve fibre layer (RNFL) as detected by an optical coherence tomography (OCT) is affected in individuals with cerebellar ataxias (CA) even in the absence of decreased visual acuity.

My hypothesis is that retinal changes in various types of inherited ataxias are more common than previously thought; that OCT may play a role in distinguishing different types of ataxias; and that RNFL structural changes have a functional correlate, and thus retinal thickness as measured by OCT has the potential to become a useful biomarker in CA.

2.2 Specific aim 1

Aim

To identify patients with genetically determined or suspected inherited ataxia, and to describe the clinical phenotypes associated with rare genetically determined ataxias.

Rationale

The inherited ataxias are highly heterogeneous neurodegenerative disorders with presentations that often overlap, and thus, distinguishing between these disorders based only on their phenotype can be difficult. Establishing a molecular diagnosis in CA is challenging and ideally entails evaluation at designated centres of expertise, providing detailed clinical evaluation and targeted investigations [25, 138]. Comprehensive clinical assessment is the fundamental first step in the diagnostic process as delineation of the clinical phenotype can guide appropriate investigations, including genetic testing. In more than a half of individuals a molecular diagnosis cannot be achieved by testing for common gene mutations using traditional methods [5]. The introduction of advanced next-generation sequencing (NGS) techniques has enabled molecular diagnosis in many previously undiagnosed cases and has led to improvement in the diagnostic yield. It has been shown that the use of NGS methods is an efficient and cost-effective way to identify a very rare and unknown genetic variants that are only present in a tiny number of individuals, thus demonstrating the potential of NGS to be informative in a single family and to assist with expanding the knowledge about the full phenotypic spectrum of rare ataxic syndromes.

With the increasing availability of NGS, thorough clinical phenotyping in a specialist clinic is not redundant, and remains critical not only to accurately select patients suitable for testing using NGS approach, but also for the interpretation of frequently detected heterozygous variants of uncertain significance [139].

2.3 Specific aim 2

Aim

To characterise the retinal findings and measure the peripapillary RNFL and macular thickness with OCT in patients with different types of inherited ataxias and to compare OCT data to age – and sex-matched healthy volunteers, who will serve as controls.

Rationale

As outlined in the previous chapter, thinning of RNFL has been documented in individuals with neurodegenerative diseases such as MSA-C, PD and Alzheimer's disease [79, 81], as well as in patients with MS with and without optic neuritis [140]. Furthermore, a modest number of studies have described retinal changes in some types of CA detected as an abnormal RNFL thinning to a degree measurable by OCT even in patients with little or no evidence of visual dysfunction [130].

In the past decade an increasing number of reports have indicated OCT's potential role as a helpful tool in the study of neurodegenerative diseases. Results obtained through OCT have demonstrated that parameters provided by this non-invasive technique are accurate to detect afferent visual pathway involvement. The literature suggests that OCT findings are highly reproducible, and thus, an attractive tool for detecting both subclinical and clinically significant optic neuropathy.

To date, among various CA, most well defined retinal findings are documented in the ARCA such as FRDA [34, 96 -98, 101] and ARSACS [91, 128], as well as in AD SCA7 [113, 114, 115, 116]. A number of case series describe the OCT features in other more

common CA [38, 105, 106, 109, 110]. However, OCT data in patients with other rarer types of CA and particularly in the often clinically and genetically overlapping highly heterogeneous HSP cohorts is still very limited.

At the present time no clear guidelines are available on whether one, several, or all of the retinal parameters measured by OCT can be used in the diagnosis of different types of CA. With phenotypic variability observed across all CA cohorts, including well characterised entities such as FRDA and ARSACS and increasingly identified *SPG7* – associated spastic ataxia among others, diagnosis can be challenging. Thus, better understanding of the possible role of the RNFL as an imaging biomarker for the measurement of change in CA may be a useful addition to the battery of assessments in individuals with different types of CA. Furthermore, RNFL changes might remain subclinical and not detected in routine clinical examination, implying the value of OCT in clinical practise.

2.4 Specific aim 3

Aim

To determine whether the pattern of retinal changes (when present) in patients with different types of inherited ataxias possesses any specificity.

Rationale

Numerous small OCT studies have demonstrated the potential value of OCT in differentiating diseases with similar clinical characteristics, such as MS and NMO [141].

In the literature, a handful of OCT studies in FRDA cohorts have reported statistically significant average RNFL thickness reduction in patients in comparison to controls with a distinctive pattern of RNFL loss; predominantly in the superior quadrant in one of the studies [34]. While average RNFL loss was also observed in larger SCA1 studies, most prominent RNFL reduction was detected in the temporal region in one of the cohorts [105]. Diffuse and sectorial, particularly temporal, RNFL loss was also reported in a small number of *SPG7* cases with optic neuropathy detected by OCT [130], similarly to findings in one *SPG55* case series [134]. In contrast, significant nasal RNFL thinning was documented in one SCA3 cohort [110]. Little is known about OCT findings in other types of ataxias.

Results from previous OCT reports suggest that FRDA is likely to involve different disease mechanisms, leading to slightly different areas of selective vulnerability in comparison to other CA [101]. However, what proportion of affected individuals demonstrates specific RNFL thinning is still unknown. In addition, the question of

whether RNFL thinning represents a rather non-specific affection in all neurodegenerative disorders or whether it might have some specificity with distinctive patterns of RNFL loss in different entities is still unanswered.

Abnormal thickening of the RNFL is a characteristic feature of ARSACS and is not seen normally in the context of chronic progressive neurodegeneration. Published OCT studies of individuals with ARSACS [91, 128] clearly demonstrate that OCT is a sensitive and specific tool, and can help to distinguish this entity from other inherited ataxias.

2.5 Specific aim 4

Aim

To determine if correlation can be found between OCT findings as an anatomical marker in individuals with different types of CA and disease severity as quantified with SARA (Scale for the Assessment and Rating of Ataxia).

Rationale

When assessing patients with CA various clinical scales have been utilised to quantify disease severity, including the International Cooperative Ataxia Rating Scale (ICARS), Friedreich's Ataxia Rating Scale (FARS) and SARA [99] (Appendix 3), while the Spastic Paraplegia Rating Scale (SPRS) has been widely used in HSP cohorts [130].

Despite considerable discrepancies in ataxia scale sizes and subscale structures, SARA total scores are significantly correlated with ICARS and FARS total scores [142]. Although originally developed for the use in ADCA, which are primarily ataxias of the cerebellar type, the literature supports successful use of SARA to assess afferent ataxia, which is the predominant form in entities such as FRDA [143]. While ICARS and FARS are more comprehensive and time consuming, SARA is easily applicable and has high interrater reliability and practicability [142].

Previous OCT studies in various CA cohorts have used different scales to quantify functional disability, but SARA score has been used relatively rarely outside the SCA studies. Only two studies to date have evaluated the correlation between peripapillary RNFL thickness and SARA score in FRDA patients [100, 101] and no studies have used

SARA to quantify disease severity in HSP cohorts. While SARA evaluates the ataxic manifestations, the SPRS, traditionally used to measure disease severity in HSP, quantifies the functional impairment of spastic paraplegia, suggesting that both scales have limitations in objectively estimating progression in the broader phenotypic spectrum of individuals with complex and overlapping CA / HSP phenotypes.

2.6 Specific aim 5

Aim

To determine if correlation can be found between OCT findings as an anatomical marker in individuals with different types of CA and disease duration.

Rationale

Published data on correlation between disease duration and thinning of the RNFL in CA are controversial and this is likely due to the differences in the cohorts studied in the context of various types of genetic ataxias. In the literature, disease duration is defined either since start of gait ataxia or since start of first symptom according to patient's report [144]. In FRDA, typically an early onset and life-shortening condition, three of five studies [34, 97, 101] which included in total over 60% of all reported FRDA patients who underwent OCT, have found correlation between RNFL and disease duration. These conflicting results may be partly explained by variations in disease duration between FRDA studies. In contrast, findings from a modest number of OCT studies in different SCAs have suggested that RNFL changes are unrelated to disease duration [105, 109, 110]. Most of the very small studies on typically slowly progressive complex HSP reported OCT findings in patients with disease duration of at least 10 years, and thus, did not answer the question whether identified optic neuropathy already existed at the earlier stages of the disease [131].

In all types of CA the rate of progression varies from person to person. Published data suggest that progression rates in different entities are not constant during the long disease

duration. For example, in the early phase of SCA2 disease, i.e. in the first 10 years of the disease, progression rates assessed with the application of clinical scales are slower than in the following years [144]. In contrast, much faster rate of progression is seen in FRDA, where other factors, such as GAA repeat length among the others, determine the speed of progression. Thus, it is likely that there is a possible association between retinal changes as detected by OCT and disease duration at least in early onset and relatively faster progressing CA with optic nerve involvement.

2.7 Specific aim 6

Aim

To document if retinal changes detected by OCT are evident over time, and thus determine the usefulness of OCT as an imaging biomarker of disease progression in patients who have had previous OCT assessment.

Rationale

Several clinical scales and quantitative performance scores have been validated and used in patients with CA to evaluate and monitor disease progression in natural history studies. Although these are adequate to measure disease severity, none has been proven to be superior for patient evaluation in much needed clinical trials.

Several potential candidates, including biological fluid compounds (cerebrospinal and serum blood biomarkers) and neurophysiological parameters have been investigated mainly in SCA3 and, although some seem to be good candidates, most of the studies were too small and / or without rigorous design for the validation of such biomarkers.

Advanced MRI techniques have also been suggested as promising neuroimaging biomarkers to monitor disease progression; however, which are the best surrogate biomarkers remains unclear. In addition, although significant, the longitudinal volumetric changes reported in one mixed SCA cohort study were relatively small [145].

In FRDA, frataxin expression, oxidative stress response pathway and metabolic biomarkers have been explored as potential biomarkers for disease progression, in addition to proposed structural (imaging and histology) and functional biomarkers of the

nervous system [146, 147, 148, 149]. Furthermore, biomarkers that reflect FRDA cardiac disease including both cardiac imaging technologies and serum biomarkers are under evaluation to assess their value as both monitoring and prognostic biomarkers [148].

With the greater recognition of subclinical phenotypic features such as optic neuropathy, and increasing availability of OCT, allowing unique morphologic visualisation of the anterior visual pathways and RNFL quantification, this method is currently explored for diagnosis and monitoring of disease progression in a variety of neurodegenerative disorders as a surrogate parameter for cerebral and / or optic nerve axonal loss. In addition, OCT is a non-invasive, quick, cost-effective, and reproducible imaging tool.

To date, only a handful of OCT studies in CA have reported RNFL changes over time and data on the utility of OCT as a potential biomarker for disease progression in CA cohorts are scarce [100, 109].

It is possible that a single biomarker is unlikely to be successful due to the complexity of the neurodegenerative processes leading to the onset and progression of CA, and that a multimodal biomarkers approach may enhance sensitivity to progression, especially when structural changes would not be detectable over short follow-up intervals in the context of slowly progressive CA.

2.8 Specific objectives

1. To perform prospective clinical and retinal evaluation of a sample of 131 individuals (128 attending the National Ataxia Clinic, Dublin, Ireland and 3 attending the Dublin Neurological Institute at the Mater Misericordiae University Hospital) with inherited CA, and 7 asymptomatic first-degree relatives of affected patients.
2. To assess patients at baseline and, when available, at an interval of at least 12 months.
3. To use clinical tools including the Scale for the Assessment and Rating of Ataxia (SARA), Snellen chart, and when appropriate, Ishihara test plate.
4. To use OCT imaging to document peripapillary RNFL and macular thickness.

Chapter 3: Subjects

3.1 Patient sources

Patients with progressive ataxia were identified from the Irish National Ataxia Clinic (NAC) in Tallaght University Hospital (TUH). Further small number of patients (three individuals) with *SPG7*-associated ataxia was recruited from the Dublin Neurological Institute at the Mater Misericordiae University Hospital. Those that fulfilled inclusion criteria were asked to participate.

Control subjects were recruited from the community as well as from the relatives that were not genetically related to patients and volunteered to participate in this study.

3.1.1 National Ataxia Clinic in Tallaght University Hospital

In a retrospective observational study we described the five-year experience at the NAC, Dublin, Ireland with evaluation of the benefits of a specialist clinic for patients with CA and how the access to commercially available advanced genetic technologies has impacted the rate of confirmed genetic diagnoses in patients with early and late-onset progressive ataxia both in familial and non-familial cases over this period of time [5].

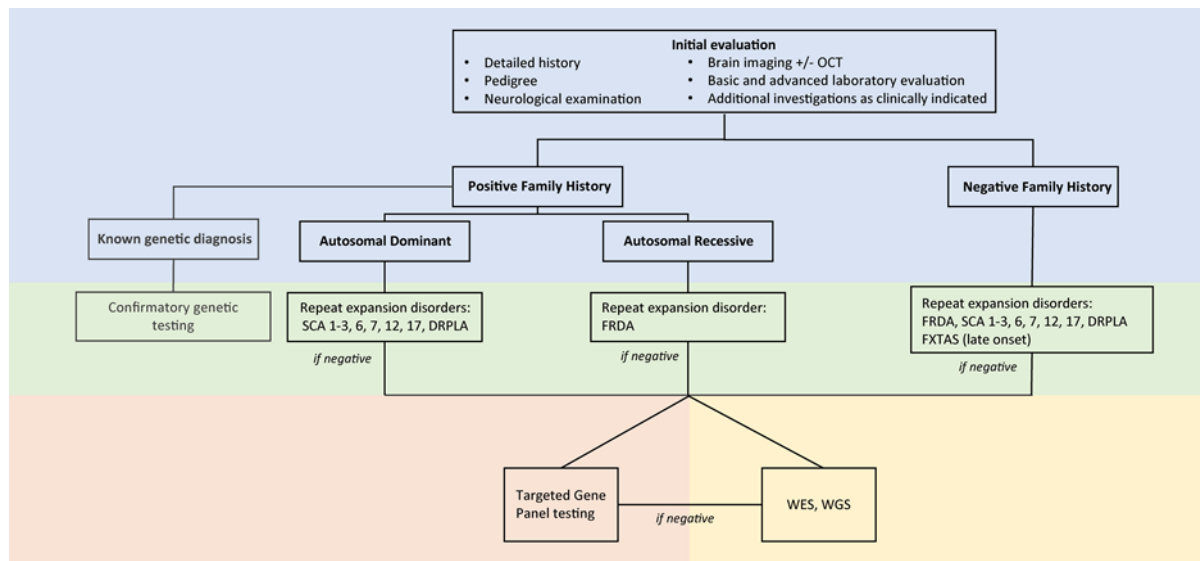
NAC is a multidisciplinary clinic run by two consultant neurologists, ataxia research fellow, ataxia nurse specialist and a cardiologist. All individuals included in the study presented between December 2014 and December 2019 with progressive ataxia as principal complaint and were not found to have an acquired non-genetic form of ataxia such as multiple system atrophy (MSA).

Clinical assessment using a standardised approach, including detailed history with age of symptom onset, speed of progression, and pedigree was performed. Each individual was clinically examined by a neurologist (Petya Bogdanova-Mihaylova, Sinéad Murphy or Richard Walsh). Basic demographics and information regarding the presenting phenotype, including cerebellar, ocular motor, pyramidal or extrapyramidal, cognitive, neuropathic, bladder and bowel dysfunction along with funduscopy findings and skeletal abnormalities were recorded in the ataxia database, established in 2014. Assessment of additional family members was performed when required for segregation analysis.

Patients had imaging with Magnetic Resonance Imaging of brain (MRI). Additional tests including nerve conduction studies, electromyography, echocardiography, optical coherence tomography, muscle and / or nerve biopsy were performed as clinically indicated.

Initial genetic testing was performed as per the algorithm shown in Figure 3.1. In individuals with a known familial diagnosis, the relevant genetic variant was tested directly.

Figure 3.1 Diagnostic algorithm for patients presenting with progressive cerebellar ataxia to the National Ataxia Clinic, TUH



Taken from [5]

Where a molecular diagnosis was not obtained following initial genetic evaluation, next-generation sequencing (NGS) testing was performed. Commercially available accredited genetic laboratories were used for targeted NGS gene panel testing (Appendix 4), whole-exome sequencing (WES) and whole-genome sequencing (WGS). Following advanced genetic testing, any variants of uncertain significance (VUS) that were identified, were discussed at multidisciplinary team meeting to achieve a consensus opinion based on careful clinical phenotyping and family analysis, where possible, as per previously published criteria [150].

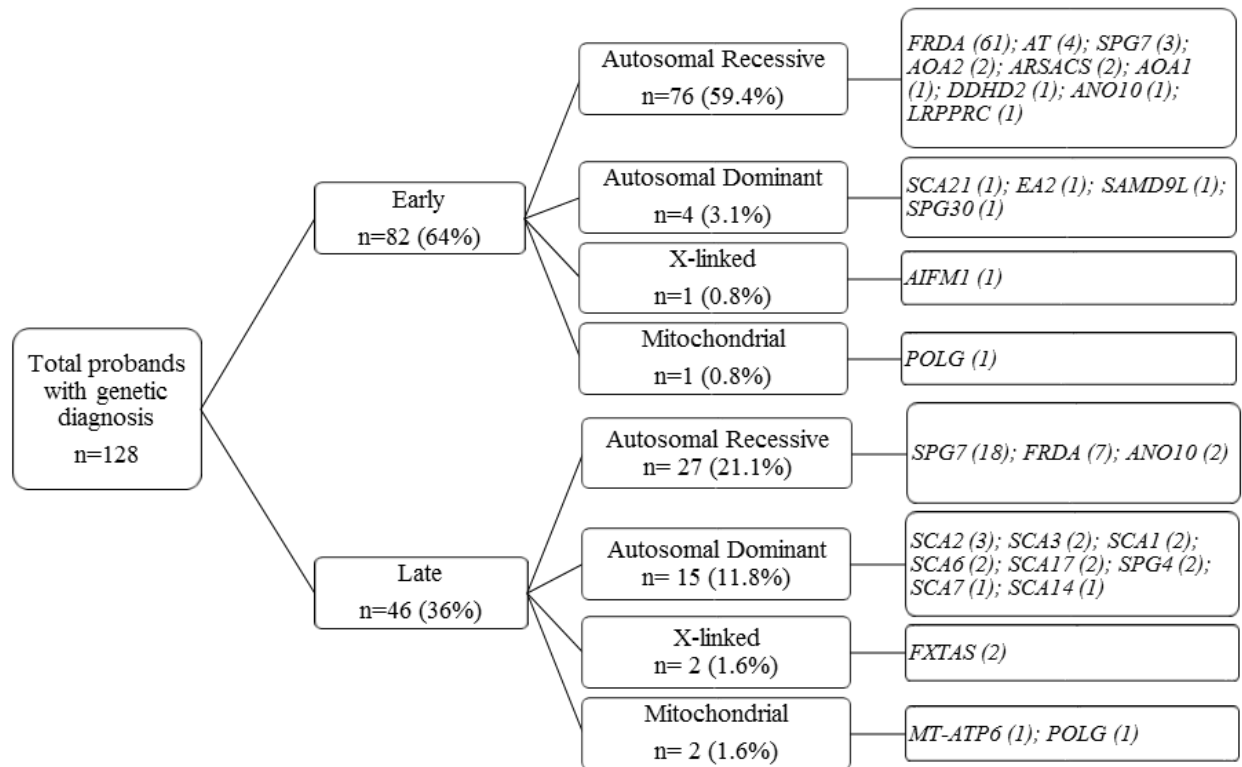
If mitochondrial disease was suspected and initial sequencing for the common mitochondrial DNA and POLG point mutations was negative, whole mitochondrial genome sequencing using blood DNA and muscle biopsy for mitochondrial analysis was performed.

3.1.2 Patient cohort

A total of 254 consecutive patients over the age of 16 years from 196 families with confirmed or suspected genetic ataxia attended the NAC between 2014 and 2019. There was a slight predominance of males (131/254, 51.6%). A small majority of individuals, 138/254 (54.3%) reported early symptom onset (<20 years). A total of 138 patients (54.5%), 80/196 probands (40.8%), had a family history of ataxia. 28 patients from 18 families had an AD pattern of inheritance, 93 patients from 56 families had an AR appearing pedigree and 8 patients from 2 families had an X-linked appearing pedigree. The majority of individuals (243/254, 95.7%) were of Irish origin. The remainder were of Asian, European and Australian ancestry.

A definite genetic diagnosis was established in 128 probands from 196 families (65.3%) accounting for a total of 178 of all 254 patients (70.1%). A molecular diagnosis was more likely in individuals with early than late symptom onset, 82/109 (75.2%) vs 46/87 (52.8%), $p=0.001$ (Figure 3.2). Similarly, a molecular diagnosis was more likely in those with a family history 63/80 (78.8%) vs those without 65/116 (56%), $p=0.001$ (Table 3.1).

Figure 3.2 Genetic diagnoses in probands with early and late onset ataxia



Taken from [5]

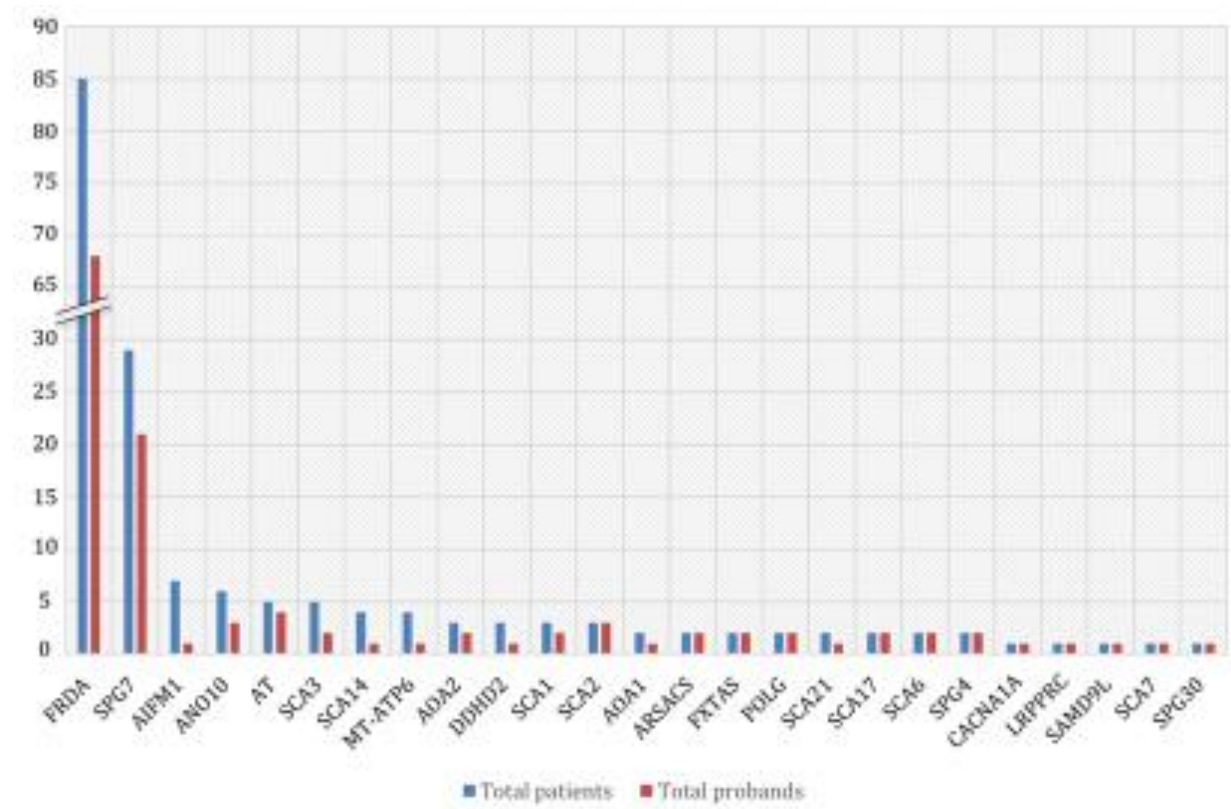
Table 3.1 Distribution of genetic causes in probands

	Familial n = 80		Sporadic n = 116	
AR	FRDA	29	FRDA	39
	SPG7	7	SPG7	14
	ANO10	2	ARSACS	2
	AT	3	ANO10	1
	DDHD2	1	AOA2	1
	AOA1	1	AT	1
	AOA2	1		
	LRPPRC	1		
AD	SCA1	2	SCA2	1
	SCA2	2	SCA17	1
	SCA3	2	EA2	1
	SCA6	2	SAMD9L	1
	SCA7	1	SPG4	1
	SCA14	1		
	SCA17	1		
	SCA21	1		
	SPG4	1		
	SPG30	1		
X-linked	FXTAS	1	FXTAS	1
	AIFM1	1		
Mitochondrial	POLG	1	POLG	1
	MT-ATP6	1		
Undiagnosed		17		51

Taken from [5]

The most common diagnosis in our cohort (in both the familial and sporadic cases) was FRDA accounting for 68 probands (34.7% of cohort), followed by *SPG7*-associated spastic ataxia (21 probands, 10.7%). The breakdown of other causes is shown in Figure 3.3.

Figure 3.3 Genetic causes of ataxia in patients attending NAC



Taken from [5]

In total, 82 probands were diagnosed following repeat expansion disorders testing; of these 59 were referred to the NAC with an established diagnosis (mainly FRDA, 91.5%) for ongoing management in a specialist clinic. In 11 individuals the diagnosis was obtained through individual gene Sanger sequencing (6/11 were referred with an established diagnosis), including AT (4), AOA1 (1), AOA2 (2), POLG (2) and SPG4 (2). Confirmatory genetic testing was performed in another 48 affected family members when the diagnosis had previously been established in the proband.

3.1.3 Diagnostic Yield

Eighty-four patients underwent testing with commercially available NGS panels. Of these, 27 had positive family history and 54/84 (64.37%) presented with late onset CA. A genetic diagnosis was made in 30/84 (35.7%). Additional 6 cases were found to have variants of potential pathogenicity in the *CACNA1A* (c.920C>T, c.539+5G>A and c.1060C>G), *SYNE1* (c.22913G>A), *SACS* (c.11380G>C and c.2825C>T), and *SPG7* (c.1781T>C) genes, warranting further investigations (currently ongoing) to clarify if findings were relevant (Table 3.2). Of those who received a definite diagnosis through NGS panel testing the majority, 21/30 (70%), had late symptom onset. The majority of positive findings, 27/30 (90%), were ARCA with the highest pick up rate for *SPG7* (n = 21, 70%). Our findings are comparable to several other studies [151, 152] with c.1529C>T (p.Ala510Val) reported as the most frequent pathogenic variant (n = 17, 81%), present in homozygous state in the majority (n = 11, 65% of families with identified c.1529C>T variant) and as a heterozygous variant in association with a second *SPG7* mutation in further 6 families (n = 7). Four individuals had heterozygous *SPG7* variants, one of them was suspected for *SPG7* and MLPA was performed but no copy number variations were found.

Table 3.2 Characteristics of patients with relevant genetic variants identified by NGS

Cases with Pathogenic and Potential Pathogenic Variants								
Cases with Known Pathogenic Variants								
NGS Method	Gene	Sex	Age/onset years	Age/testing Years	Family history	Phenotype	Nucleotide change	Protein change
	SPG7	M	50	67	Sporadic	ATX, UMN	c.1053dup c.1045G>A	p.Gly352fs p.Gly349Ser
		M	15	46	Sporadic	ATX, UMN	c.1529C>T c.1053dupC	p.Ala510Val p.Gly352fs
		M	35	53	Affected sibling	ATX, UMN	Hom c.1529C>T	Hom p.Ala510Val
		M	42	49	Sporadic	ATX, UMN	Hom c.1529C>T	Hom p.Ala510Val
		M	48	66	Sporadic	ATX, UMN	Hom c.1529C>T	Hom p.Ala510Val
		M	51	74	Affected sibling	ATX, UMN, CPEO, Ptosis	c.1192C>T c.2246C>T ^a	p.Arg398* p.Pro749Leu ^a
		M	12	54	Sporadic	ATX, UMN	c.1529C>T c.850_851delinsC	p.Ala510Val p.Phe284fs
		M	32	44	Sporadic	ATX, UMN	Hom c.1529C>T	Hom p.Ala510Val
		F	28	63	Affected sibling	ATX, UMN	Hom c.1529C>T	Hom p.Ala510Val
		M	55	60	Sporadic	ATX, UMN	Hom c.1529C>T	Hom p.Ala510Val
		M	30	48	Affected siblings	ATX, UMN	c. 1049_1077del ^a c. 1715C>T	p.Pro350Glnfs*36 ^a p.Ala572Val
		M	50	64	Sporadic	ATX, UMN	Hom c.1529C>T	Hom p.Ala510Val
		F	20	39	Sporadic	ATX, UMN	Hom c. 1715C>T	Hom p.Ala572Val
		M	50	68	Affected siblings	ATX, UMN	Hom c.1529C>T	Hom p.Ala510Val
		F	42	56	Affected sibling	ATX, UMN	c. 1529C>T c. 2084T>C	p.Ala510Val p.Leu695Pro
		M	33	42	Sporadic	ATX, UMN	Hom c.1529C>T	Hom p.Ala510Val
		F	42	53	Sporadic	ATX, UMN	c. 1529C>T c.1939del ^a	p.Ala510Val p.Ala647fs ^a
		M	34	73	Sporadic	ATX, UMN	Hom c.1529C>T	Hom p.Ala510Val
		F	43	52	Sporadic	ATX, UMN	c. 1529C>T c.2228T>C	p.Ala510Val p.Ile743Thr
		M	53	61	Sporadic	ATX, UMN	Hom c.1529C>T	Hom p.Ala510Val
		M	20	45	Affected sibling	ATX, UMN, Spasmodic dysphonia	c.861dup c.1529C>T	p.Asn288* p.Ala510Val
	ANO10	F	43	61	Affected siblings	ATX, UMN, TA, COG	Hom c.132dupA	Hom p.Asp45fs
		F	9	70	Affected sibling	ATX, UMN, MJ, COG	c.132dupA c.96delA	p.Asp45fs p.Glu33fs
		M	24	38	Sporadic	ATX, UMN	Hom c.132dupA	Hom p.Asp45fs

WES	SACS	M	2	25	Sporadic	ATX, UMN, NP, WMC	c.[10907G>A;10954C>A] c.4453G>A ^a	p.[Arg363Gln; Pro3652Thr] p.Ala1485Thr ^a
		F	infancy	16	Sporadic	ATX, UMN, COG, NP	Hom c. 3328dup	Hom p.Ile1110fs
	DDHD2	F	12	55	Affected siblings	OMA, ID, NP, TCC	Hom c.1891+2 T>C ^a	p.? ^a
	TMEM240	M	18	76	Sporadic	ATX, UMN, PSY, COG	c.509C>T	p. Pro170Leu
	KIF1A	M	3	15	Affected parent	UMN, NP	c.206C>T	p.Ser69Leu
	CACNA1A	F	10	27	Sporadic	Episodic ATX	c.5266C>T	p.Arg1756Trp
	SAMD9L	F	12	62	Sporadic	ATX, NP,TA	c.2956C>T	p.Arg986Gs
	MT-ATP6	M	24	31	Affected mother+siblings	ATX, NP,Ptois	m.8851T>C	p.ATP6: (Trp109Arg)
	LRPPRC	F	4	22	Affected sibling	ATX,ID	(?_44200513)_(44209723_?)del c.2858A>T ^a	(?_44200513)_(44209723_?)del g.44152244T>A ^a
	PRKCG	M	39	43	Affected parent, siblings,children	ATX	c.220 C>T	p.H74Y
WGS	AIFM1	M	1	37	Affected relatives (males)	ATX,NP,Deafness	c.1019T>C	p.Met304Thr
Cases with Potential Pathogenic Variants								
NGS panel	CACNA1A	M	9	49	Sporadic	Episodic ATX	c.920C>T ^b	p.Ala307Val ^b
	CACNA1A	F	29	43	Sporadic	ATX	c.539+5G>A ^b	p.? ^b
	SYNE1	F	16	58	Sporadic	ATX,NP,COG	c.5844C>A c.22913G>A ^c	p.Cys1948* p.Gly7638Asp ^c
	SACS	F	5	56	Sporadic	ATX,UMN	c.11380G>C ^b c.2825C>T ^b	p.(Val3794Leu) ^b p.(Thr942Ile) ^b
	SPG7	M	61	66	Sporadic	ATX	c. 1529C>T c.1781T>C ^c	p.Ala510Val p.Val594Ala ^c
	CACNA1A	M	1	25	Affected sibling + nephew	ATX,UMN,COG	c.1060C>G ^d	p.Leu354Val ^d
WGS	WASHC5	F	41	58	Sporadic	ATX,UMN	c.3104G>A ^c	p.Arg1035His ^c

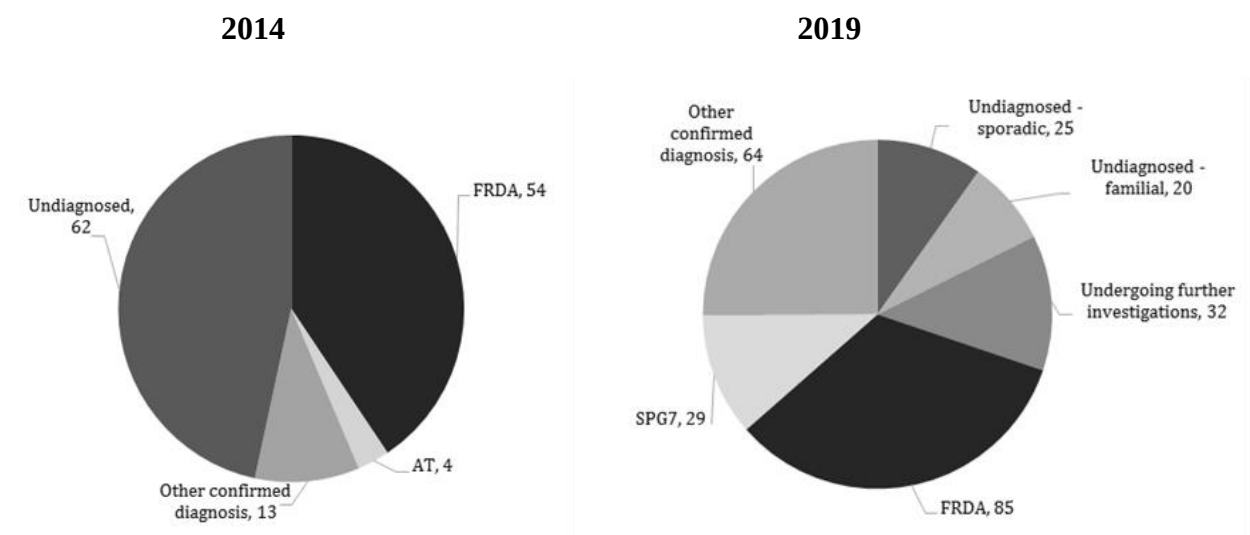
M Male, F Female, ATX Ataxia, UMN Upper motor neuron features; OMA Oculomotor apraxia, TA Telangiectasia, NP Neuropathy, MJ Myoclonic jerks, ID Intellectual disability, PSY Psychiatric symptoms, CPEO Chronic progressive external ophthalmoplegia, COG Cognitive impairment, WMC white matter changes on brain magnetic resonance imaging, TCC Thin corpus callosum, ^a Novel variant, ^b Potential pathogenic, ^c Potential pathogenic, awaiting segregation analysis, ^d Potential pathogenic, phenotype inconsistent

Taken from [5]

Twenty patients had WES and a definitive diagnosis was obtained in 4 (20%). WGS was diagnostic in 1/5 probands tested, identifying a pathogenic mutation in the *AIFM1* gene and further allowing the diagnosis in 6 other similarly affected family members following confirmatory carrier testing [153]. One further individual has a VUS in the *WASHC5* gene (c.3104G>A) for which further investigations are ongoing (Table 3.2).

As demonstrated in Figure 3.4, the diagnostic success rate in the ataxia clinic population as it stood in 2014 was 53% (71/133). At that time the main diagnoses were FRDA (54/133, 40.6%) and AT (4/133, 3%). In 2019, using a combination of single gene sequencing and NGS in an expanded clinic cohort, we achieved an overall diagnostic rate of 70.1% (178/254).

Figure 3.4 Distribution of genetic diagnoses in patients in 2014 and 2019



Taken from [5]

3.1.4 Comparisons with other cohorts

In this study, we evaluated a large cohort of patients attending the NAC, the only clinic in Ireland dedicated to the investigation and management of inherited CA, over a five year period. We reported a notable improvement in the rate of genetic diagnoses using commercially available genetic testing, with a molecular diagnosis achieved in 65.3% of probands [5]. In addition, a further 7 cases (5.5%) have VUS considered potentially pathogenic. This diagnostic yield is higher than previously reported from other tertiary referral centres. In an Australian cohort of suspected genetic ataxia patients, detection rate was 28.8% [154]. A diagnostic yield of 63% was documented in a large UK cohort; however, this study included individuals with MSA, immune-mediated and other acquired ataxias, with gluten ataxia reported as the commonest cause in their sporadic patients [25]. A French study documented an overall diagnostic rate of 65% (52/80) among sporadic progressive CA individuals with onset after 40 years of age but only 11% had genetic diagnosis [4]. Our higher genetic diagnostic yield is likely to reflect the inclusion of early onset and familial cases and exclusion of acquired causes. In our subspecialty quaternary referral clinic it is likely that most individuals have already had the most common acquired causes excluded. Furthermore, in addition to NGS panel testing we also availed of WES and WGS and this has likely contributed to the higher rate of genetic diagnoses.

As anticipated, we demonstrated that patients most likely to receive a genetic diagnosis are those with a family history and those with early symptom onset, similarly to other studies [25, 155, 156]. Within our cohort, ARCA was most common with 53% of probands having FRDA, followed by *SPG7*-associated spastic ataxia (16.4%) and these findings are in keeping with other studies [12, 25] confirming FRDA as the most common ARCA. In contrast to the UK, where SCA6 is the commonest ADCA [25], in

our cohort amongst ADCAs, SCA3 was the most frequent diagnosis. Similarly, EA2 was found to be much more common in the UK than in Ireland [25]. Interestingly, in 2014 prior to widespread clinical use of NGS no patients had been diagnosed with *SPG7*, *ANO10*, *AIFM1*, *DDHD2* or other rare ataxia genes. These findings highlight the underlying genotypic heterogeneity of the CAs in Ireland and change our knowledge about the prevalence of CA subtypes.

The main reason for the improved diagnostic yield over the five year period is the use of NGS techniques. These have become increasingly available and cost-effective. Before the era of NGS, where only testing for repeat expansion disorders and sequential Sanger sequencing of individual genes was possible, the diagnostic yield was 33% in late-onset CA across a UK population-based cohort [157], with an even lower diagnostic rate of 13.8% in an Australian cohort [154]. In our clinic the detection rate for repeat expansions was much higher, 48%, and in contrast to other studies the most common molecular diagnosis identified was FRDA [154, 157]. However, similar to other reports [25], in our NAC cohort we have included both individuals with early and late symptom onset, which can explain the highest prevalence of FRDA, typically an early onset progressive ataxia. Furthermore, Ireland has been reported to have one of the highest observed FRDA prevalence [15], which also contributes to the discrepancy in the detection rate of FRDA cases among different cohorts.

NGS gene panels provided the diagnosis in over 35% of probands with negative repeat expansion testing. A further 7% were found to have variants of potential pathogenicity warranting further investigation. This detection rate is higher than previously reported yield of 18-34.4% using NGS targeted panels in CA [25, 154, 155, 158, 159]. In contrast to other studies [155, 158], in our cohort both individuals with and without family history, and with symptom onset at any age were tested. The majority of our probands

were extensively investigated before NGS panel testing with most common genetic causes of cerebellar ataxia already ruled out, contrary to other studies, where common SCAs were not excluded prior to targeted panel testing [158]. Paraplegin (*SPG7*), a gene with emerging importance in inherited spastic-ataxia was not included in all reported studies, which has likely contributed to their lower detection rate [158]. Interestingly, we have identified *SPG7* not only as the most common “new” genetic ataxia in our cohort (first index case genetically confirmed in January 2015), but also as the second most common diagnosis in our clinic, partly filling the large diagnostic gap that has existed between the relatively more prevalent FRDA and the other ARCAs which are far less common such as AT and AOA. There were no known cases with *SPG7* in 2014; until cerebellar ataxia was recognised as a core clinical disease feature of *SPG7*-associated disease [160], genetic testing for mutations in paraplegin was not considered in patients with late onset unexplained ataxia. Our findings indicate a high frequency of *SPG7*-associated spastic ataxia in Ireland which supports previous communications that *SPG7* mutations account for a significant proportion of progressive ataxia worldwide [16, 151]. Characteristic phenotype reported by the Sheffield Ataxia centre consisting of prominent dysarthria, mild spasticity and proximal muscle weakness resulting in a waddling gait [25, 161] was not consistently observed in our clinically heterogeneous *SPG7* subgroup. Furthermore, as the non-specific phenotypic features in most cases cannot reliably predict the genotype, the suggested direct screening for *SPG7* mutations was not felt to be appropriate for the cases in our cohort.

The evolution of the progressive stepwise approach to obtaining a genetic diagnosis in ataxic syndromes has led to a new and interesting cohort of patients who do not have a mutation in genes commonly associated with ataxia, or so-called ‘panel-negative’ patients. In our clinic population, nearly a third of the “panel-negative” probands

underwent WES with pathogenic variants identified in 20%, similar to previous reports [150, 156, 162, 163, 164]. The percentage of confirmed disease-causing mutations varies widely in the literature, between 21-60% depending on criteria used; however, some of these studies only excluded repeat disorders before proceeding to WES and others include VUS in their yield [150, 156, 162 - 165]. In contrast to prior studies, in our cohort detection rate of pathogenic variants through WES (4/20) was higher in individuals with a family history.

Only two prior studies have investigated the yield of WGS in individuals with progressive ataxia. Kang et al performed WGS in 3 patients with ataxia, reporting a yield of 33.3% [154] while Kim et al proceeded to WGS in 18 patients with spastic paraplegia with / without ataxia after excluding SPG4, ATL1 and common SCAs to demonstrate a yield of 38.9% [166]. However the yield of WGS reported in larger cohorts of patients with other movement disorders is generally lower [167].

In addition to improved diagnostic rates, one of the benefits of high-throughput NGS methods is their potential to reduce the cost-to-diagnosis ratio [168, 169]. Albeit the price of NGS testing is decreasing, these methods still remain expensive and require interpretation therefore should be only requested by specialists with expertise in an appropriate setting, such as in dedicated ataxia clinics [168]. We have previously demonstrated that NGS-based panel approach is cost-effective when compared to the expenditure on traditional single gene testing [139]. The cost-effectiveness of NGS gene panel testing and WES has been further shown in paediatric muscle diseases [170]. Although WGS offers sequencing of the entire autosomal DNA and thus also covers non-exon areas where an increasing number of causative mutations for neurological disorders are being identified, this is the most expensive of the commercially available

NGS techniques, with the current cost increased almost three-fold in comparison to WES.

Cost of WES has reduced over the last 5 years and is currently lower than the cost of NGS panel testing in addition to improved coverage than previously. Unlike NGS panel testing, which is limited to a set of genes known to commonly cause ataxia, WES analyses coding regions of all genes, leading to increased diagnostic potential and identification of mutations in genes not hitherto known to cause ataxia, thereby expanding knowledge of the phenotype. If all individuals in our cohort who did not have repeat expansions were tested using WES directly without panel testing, the overall cost would have reduced significantly, but the detection rate would have likely remained the same. Despite the challenges of interpreting frequently detected VUS and additional incidental findings associated with WES, we suggest that WES should be considered as the NGS method of choice in individuals negative for repeat expansions as a more efficient and cost-effective approach. Our experience shows that when VUS are detected, deep clinical phenotyping in a specialist clinic in a critical way is crucial for the interpretation of results and consideration of additional diagnostic steps, including family segregation or functional studies.

Despite extensive investigations, our approach in evaluation of individuals with progressive ataxia, and recent advances in genetics, nearly one third of our patients still remain genetically undiagnosed. For some individuals who underwent NGS, pathogenic variants might have been missed or might be localised outside analysed regions. The aetiology in the “panel-negative” subgroup is likely to be heterogeneous. Mitochondrial DNA sequencing remains complex and with the relatively high rate of presentations resulting from defective DNA repair caused by mutations in mitochondrial genes, a proportion of our cohort might represent undiagnosed mitochondrial disease. It is also

possible that some of the undiagnosed individuals might have ataxia associated with epigenetic processes which are currently unrecognised. Although most common repeat expansion disorders were ruled out in this subgroup of patients, some rarer types are challenging to identify and might be considered as potential underlying cause. A number of individuals attending our clinic display a combination of late onset CA, neuropathy and vestibular impairment, or CANVAS, a common cause of adult onset ataxia [171]. Repeat expansions in *RFC1* gene and other as yet unidentified genes, may account for some of the undiagnosed group and in this cohort a proportion of patients with negative NGS testing and those with phenotype suggestive of CANVAS had genetic testing for *RFC1* repeat expansions.

The diagnostic rate in this Irish cohort of patients with progressive ataxia has improved significantly with the establishment of a dedicated ataxia clinic allowing for specialist assessment in addition to enhanced access to NGS technologies. This has led to an increased diagnostic yield in many familial and sporadic cases where traditional methods were unsuccessful. The prevalence of different subtypes of inherited ataxias has changed significantly and although FRDA remains the most frequent CA in our cohort, certain non-FRDA ataxias, particularly *SPG7*–associated spastic ataxia and *ANO10*–associated phenotype now outnumber AT and AOA. We anticipate that further expansion in our ability to detect genetic factors contributing to familial ataxia and informative evaluation of similar cohorts globally will assist in filling in the remaining gaps in this rapidly evolving story.

3.2 Inclusion criteria

- Age >16 years
- Genetically determined ataxia
- Suspected inherited ataxia without a genetic diagnosis
- Relatives of individuals either with known or suspected inherited ataxia, who are genetically related to the affected individuals (seen when required for segregation analysis)
- Ability to understand and sign an informed consent

3.3 Exclusion criteria

- Acquired non-genetic form of ataxia such as MSA
- High myopia
- Unwillingness or inability to take part in the study
- Lack of capacity to consent

Chapter 4: Methods

4.1 Clinical assessment

Clinical assessment using a standardised approach, including detailed history with age of symptom onset, speed of progression, disease duration and pedigree was performed and each individual had comprehensive neurological examination. Basic demographics and information regarding the presenting phenotype, inclusive of cerebellar, ocular motor, pyramidal, extrapyramidal, cognitive, neuropathic, bladder and bowel dysfunction along with fundoscopic findings and skeletal abnormalities were recorded in the ataxia database, established in 2014.

4.2 Initial diagnostic evaluation

As outlined in Chapter 3, to exclude common acquired aetiologies a number of blood tests were performed (Table 4.1). Patients had routine clinical 1.5-T Magnetic Resonance Imaging of the brain (MRI). Additional tests including nerve conduction studies, electromyography, echocardiography, autonomic testing, muscle and / or nerve biopsy were performed as clinically indicated.

Table 4.1 Basic and common advanced laboratory evaluation

FBC, blood film	Vitamin E	Methylmalonic acid
ESR	Thyroid antibodies	Homocysteine
Vit B12, Folate	Angiotensin converting enzyme	Lyme Disease screening
Glucose	Anti-GAD antibodies	HIV screening
HbA1C	Paraneoplastic antibodies	VDRL / TPHA or PRP test
U&Es	Anti-VGKC antibodies	CK
LFTs	anti-VGCC antibodies	Cholesterol
TFTs	SSA/SSB antibodies	Urine and serum amino acids
Antinuclear antibodies	Anti-gliadin antibodies	Urine and serum organic acids
Fasting lipids	α fetoprotein	Urine protein electrophoresis
SPEP	Peripheral blood for acanthocytes	Plasma Oxysterol
	Copper, Ceruloplasmin, 24 h urine copper	Very long chain fatty acids
	Lactate, Pyruvate	Beta Hexosaminidase A and B
	CSF studies	Phytanic acid

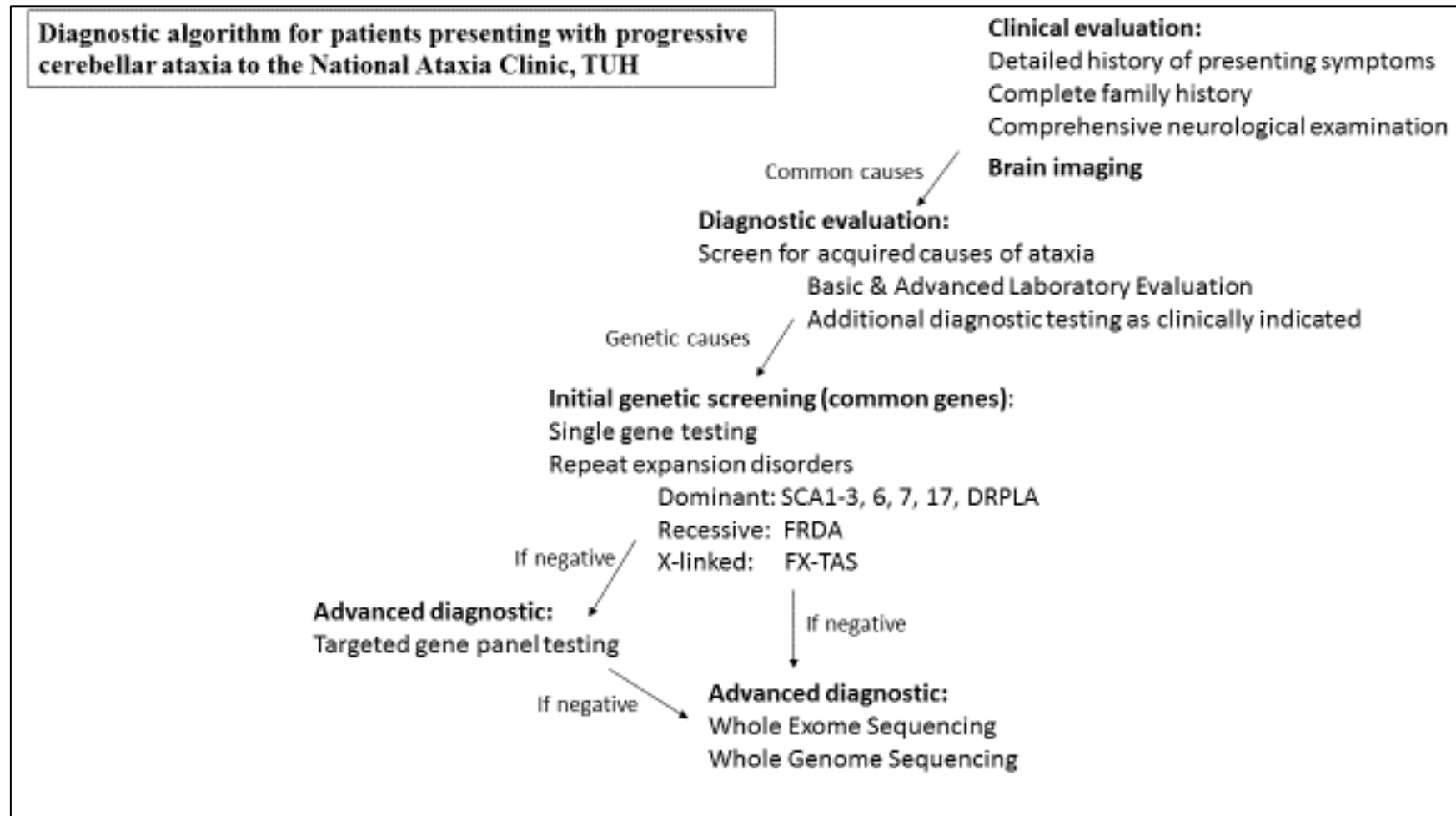
4.3 Genetic testing

Bloods for initial genetic testing were taken in TUH, usually following patients clinic visit, and send to the Department of Clinical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin as per the Instructions for Submitting Samples for Genetic Testing. As described in Chapter 3, initial genetic testing was performed as per the algorithm shown in Figure 4.1. In individuals with a known familial diagnosis, the relevant genetic variant was tested directly.

Where a molecular diagnosis was not obtained following initial genetic evaluation, commercial next-generation sequencing (NGS) testing was performed, including targeted NGS gene panel testing, whole-exome sequencing (WES) and whole-genome sequencing (WGS) (Appendix 4). Variants of uncertain significance (VUS) that were identified on advanced genetic testing were discussed at multidisciplinary team meeting to achieve a consensus opinion based on careful clinical phenotyping and family analysis where possible as per previously published criteria [150] and according to the American College of Medical Genetics and Genomics (ACMG) guidelines [172].

If mitochondrial disease was suspected and initial sequencing for the common mitochondrial DNA and POLG point mutations was negative, whole mitochondrial genome sequencing using blood DNA and muscle biopsy for mitochondrial analysis was performed.

Figure 4.1 Diagnostic algorithm for patients presenting with progressive cerebellar ataxia to the National Ataxia Clinic, TUH



4.4 Location of assessment

After initial evaluation in clinic, patients who agreed to participate were offered an appointment in the Raymond P Murphy Neurology Assessment and Research Unit (NARU) of TUH, which was opened in 2016.



4.5 Scale for the Assessment and Rating of Ataxia

All patients were evaluated with the Scale for the Assessment and Rating of Ataxia (SARA) [99] (Appendix 3) to assess disease severity.

Equipment required includes a stopwatch and an examination bed.

The SARA, consisting of eight quantitative examination features for gait, stance, sitting, speech disturbance, and limb kinetic functions, yields a composite ataxia score in the range of 0 (no ataxia) to 40 (most severe ataxia). The individual item scores are as follows: Gait (0 to 8), Stance (0 to 6), Sitting (0 to 4), Speech (0 to 6), Finger chase (0 to 4), Nose-finger test (0 to 4), Fast alternating hand movements (0 to 4), and Heel-shin slide (0 to 4).

Each category was assessed as described below.

4.5.1 Gait was assessed without shoes. Patient was asked 1) to walk at a safe distance parallel to a wall including a half-turn (turn around to face the opposite direction of gait) and 2) to walk in tandem (heels to toes) without support.

4.5.2 Stance was assessed without shoes, eyes open. Patient was asked to stand 1) in natural position 2) with feet together in parallel (big toes touching each other) and 3) in tandem (both feet on one line, no space between heel and toe). For each condition, three trials were allowed and best trial was rated.

4.5.3 Sitting. Patient was asked to sit on an examination bed without support of feet, eyes open and arms outstretched to the front.

4.5.4 Speech was assessed during normal conversation.

4.5.5 Finger chase. Patient was asked to sit comfortably for steps 4.5.5 to 4.5.7. If necessary, support of feet and trunk was allowed. Examiner sat in front of participant and performed 5 consecutive sudden and fast pointing movements in unpredictable directions in a frontal plane, at about 50% of participant's reach with an amplitude of 30cm and a frequency of 1 movement every 2s. Participant was asked to follow the movements with their index finger, as fast and precisely as possible. Average performance of last 3 movements was rated.

4.5.6 Nose-finger test. Patient was asked to point repeatedly with their index finger from their nose to examiner's finger which was in front of the participant at about 90% of their reach. Movements were performed at moderate speed. Average performance of movements was rated according to the amplitude of the kinetic tremor. Dysmetria was not rated.

4.5.7 Fast alternating hand movements. Patient was asked to perform 10 cycles of repetitive alternation of pro - and supinations of the hand on their thigh as fast and as precisely as possible. Movement was demonstrated by examiner at a speed of approximately 10 cycles within 7s. Exact times for movement execution were taken.

4.5.8 Heel-shin slide. Patient lied on examination bed, without sight of their legs and was asked to lift one leg, point with the heel to the opposite knee, slide down along the shin to the ankle, and lay the leg back on the examination bed. The task was performed 3 times. If patient slid down without contact to shin in all three trials this was rated 4.

Limb kinetic functions (items 4.5.5 to 4.5.8) were rated separately for each side and the arithmetic mean $((R+L)/2)$ was included in total score. Once each of the 8 categories had been assessed, the total was calculated to determine the severity of ataxia.

4.6 Visual acuity

Visual acuity (VA) was measured from a standard distance using a standard Snellen eye chart with a white background (Appendix 5). Good natural light or adequate illumination was ensured. Participant was positioned (sitting or standing) at a standard distance from chart. Each eye was tested separately, at first without any glasses (if worn). Patient was asked to cover the left eye with their hand to completely block the vision of the covered eye. If the patient was unable to cover their eye (e.g. upper limb weakness or severe incoordination), an assistance was sought from a nurse or a relative in attendance. If glasses were worn, the hand went over the top of the glasses. Patient was asked to avoid pressure on the eye as it might affect the eye's vision when tested. Patient was asked to read from the top of the chart and from left to right and continue reading the letters on each successively smaller line until they cannot read the line clearly and start to make multiple errors.

Visual acuity was recorded for each eye as a fraction, e.g. 20/20 and the smallest line read was documented.

Same steps were then repeated on the other side.

If the patient could not read the largest (top) letter from standard distance, they were moved closer, 1 metre at a time, until the top letter could be seen. If the top letter could not be read, the patient was asked to count how many examiner's fingers hold up at varying distance of less than 1 metre they could see. This was recorded as Count Fingers (CF).

If the patient could not count fingers, the examiner moved their hand and checked if the proband could see a moving hand. This was recorded as Hand Movements (HM). If the patient could not see hand movements, the examiner shined a torch toward the eye. If

the patient could see the light, this was recorded as 'perception of light' (VA = PL). If the patient was unable to perceive light, this was recorded as 'no light perception' (VA = NLP).

After testing without any correction, the patient was tested while wearing their current distance glasses in each eye separately, with correction.

If 20/20 line (normal vision) was not achieved, pinholes were used to see if vision improved for each eye separately.

VA results were expressed in logarithm of the minimal angle of resolution (LogMAR) for analysis.

4.7 Colour vision

Colour vision testing was performed in selected patients, using the Ishihara plate test, 24 plates Edition [173].

Participant was asked to wear the vision aids that he / she is normally required to wear. Tinted lenses were not permitted since they alter colour vision. The examiner (PBM) was screened and classified as having normal colour vision prior to testing others. Good natural light or adequate illumination was ensured. Both eyes were tested simultaneously.

The test plate was hand held or placed on a table at 'arm's length', approximately 66 cm from the eyes. Patient was asked to 'Tell me the numbers that you can see as I turn the pages. If you don't see a number then I will turn to the next page'. About four seconds were allowed for each plate.

Answers were recorded. If 13 or more plates were read normally, the colour vision was regarded as normal. If only 9 or less plates were read normally, the colour vision was regarded as deficient.

4.8 Optical coherence tomography

4.8.1 OCT instrumentation

All participants in the study underwent spectral-domain optical coherence tomography (SD-OCT) examination with commercially available Topcon 3D OCT-2000 to measure the RNFL and macular thickness (Figure 4.2).

The Topcon OCT 2000 features 3D imaging, and presents a wide scan. The Topcon OCT 2000 is relatively easy to use and most of the functions are virtually automated, requiring minimal interference by operators during the assessment. This machine features automatic disc searches and centre disc detection, as well as fovea centre detection. The model also has a joystick controller which is motorised for high efficiency. The joystick controller allows for non-distracted adjustment, which enables the operator to make precision movements without looking away from the screen.

Figure 4.2 Topcon 3D OCT-2000



To ensure that the risk for infections was minimal, the OCT machine was cleaned after each participant.

4.8.2 Scan acquisition

OCT was performed in a darkened room (NARU). Images were acquired in the seated position with the subjects facing the OCT equipment. After registering the participant, a scanning pattern from the custom options available was selected. Throughout scanning, participants were instructed to fixate on an internal green target provided by the equipment.

Both eyes of each participant were scanned using two standard acquisition protocols with a scanned area of 6-mm cube: macular (6.0 x 6.0 mm, 512 x 128) and 3D optic disc (6.0 × 6.0 mm, 512 × 128). In subjects with involuntary eye movements repeated scans were required in order to obtain scans without eye movement artefacts.

When the pupil diameter of the participant was small (pupil diameter $\leq 4.0\text{mm}$), the small pupil diaphragm selector button was set to ON to change the diaphragm.

If the image remained of poor quality, then mydriasis with local eye drops (Tropicamide 1%) was applied.

In a small number of cases, imaging of both eyes was not possible due to technical reasons, including eye movement artefacts or failure to achieve acceptable image quality. Scans with poor image quality, defined as scans with an image quality score of $\leq 50/100$ were excluded from analysis.

In each case results were evaluated after the examination was complete.

4.8.3 OCT parameters

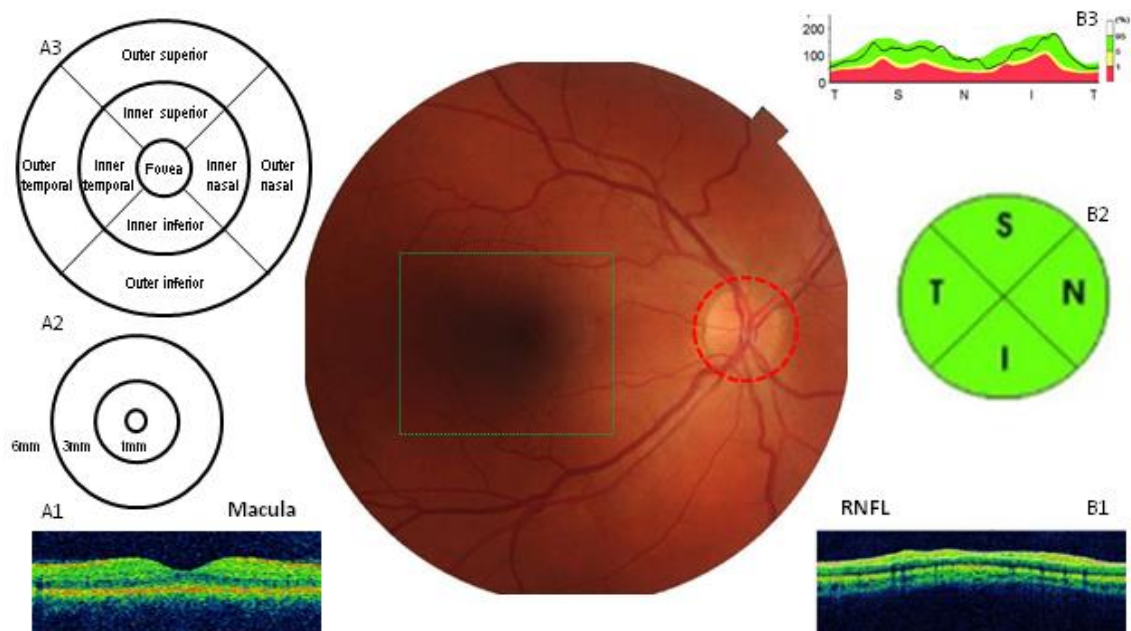
For the RNFL analysis, an optic disc cube of data centred in the optic nerve head were acquired. Evaluated peripapillary RNFL thickness parameters included average thickness of a 360° measurement, and the thickness in each quadrant around the disc (Figure 4.3): temporal (316° to 45°), nasal (136° to 225°), superior (46° to 135°), and inferior (226° to 315°).

The macula thickness parameters evaluated included overall thickness and foveal thickness. According to the Early Treatment Diabetic Retinopathy Study (ETDRS) grid [174], macula is divided into 9 regions with 3 concentric rings measuring 1 mm (innermost ring), 3 mm (inner ring) and 6 mm in diameter (outer ring) centred on the fovea (Figure 4.3); the 3 mm inner ring and 6 mm outer ring are further divided into four equal regions.

The foveal thickness was defined as macular thickness within the innermost 1 mm ring of the ETDRS map, while mean macular thickness was defined as the average macular thickness from all 9 regions of ETDRS map.

Macular superior (46° to 135°), inferior (226° to 315°), nasal (136° to 225°), and temporal (316° to 45°) quadrants thickness data was recorded but not analysed.

Figure 4.3 Macular and RNFL thickness analysis



Macular analysis (Left), showing Horizontal Tomogram (A1), and standard ETDRS map: map diameters centred on the fovea (A2) and 9 standard ETDRS regions (A3). RNFL analysis (Right), showing Horizontal Tomogram (B1), Pie graph of quadrants (B2), Quadrants: T = temporal; S = superior; N = nasal; I = inferior, and circular tomogram (B3) representing quantitative analysis of RNFL thickness (black line) and normative data set (green area = 95% confidence interval, yellow area = 99% CI, red area = outside 99% CI).

Following scan acquisition, the macular and RNFL measurements were automatically calculated by OCT using the existing software.

Normative data for the Spectral-Domain OCT examination with Topcon 3D OCT-2000 (SD-OCT) were provided by a study of 189 healthy individuals with age range between 19–84 years [175].

4.9 Timing of assessment

4.9.1 Initial assessment

Baseline assessment of majority of the patients was performed at a different time from the neurological assessment in clinic, however, some individuals had initial assessment on the same day as clinic visit. Initial assessment consisted of clinical evaluation of ataxia severity using the SARA score, and visual acuity using the Snellen chart, followed by the OCT. The entire assessment took approximately 45 minutes to complete.

4.9.2 Follow-up assessment

Patients who had follow-up visits annually underwent clinical evaluation of ataxia severity using the SARA score, visual acuity using the Snellen chart, as already described, and the OCT.

4.10 Statistical analysis

Statistical analysis was undertaken using Microsoft Excel (2013) and IBM® SPSS® Statistics for Windows version 25.0.

For each subject, the OCT measurements were averaged across both (right and left) eyes. In cases where measurements from one eye were invalid for technical reasons or ocular abnormalities, the value for only the eye with the valid measurement was used.

Patients with FRDA and *SPG7*-associated spastic ataxia were compared with healthy controls. Demographic differences between patients and controls were analysed using an independent sample Mann-Whitney U test. Comparisons between OCT measurements of controls and each of the patients groups were performed using parametric statistics. For continuous variables, student's t-test was used to compare means between two groups and ANOVA was used to make comparisons between multiple groups, followed by a post-hoc analysis.

To determine whether RNFL thickness and foveal thickness correlated with the SARA score and disease duration, Pearson's correlation test and Spearman's rank correlation test were used.

Changes in RNFL thickness from baseline were assessed in patients who had at least one follow-up visit using Wilcoxon-signed rank test, accounting for expected minimal age-related RNFL thinning [61].

Descriptive statistics were used to summarise the characteristics of other types of inherited ataxia groups.

A p value <0.05 was considered statistically significant in all analyses.

4.11 Ethics approval

The study was approved by the joint Tallaght University Hospital and St. James' Hospital Research Ethics committee. Informed consent was obtained from all participants before inclusion in the study. The aims of the study and examination required were explained to participants who were given an information sheet (Appendix 6). They had time and opportunity to ask questions before signing consent form (Appendix 7).

Chapter 5: Clinical phenotype and optical coherence tomography in Friedreich's ataxia

5.1 Introduction

5.1.1 Friedreich's ataxia

Friedreich's ataxia (FRDA) is a slowly progressive neurodegenerative disorder affecting both the central and the peripheral nervous systems. Degeneration of the dorsal root ganglia and spinal roots, dorsal columns, spinocerebellar and corticospinal tracts, and cerebellar dentate nucleus leads to the clinical picture of progressive limb and gait ataxia, proprioceptive loss, absent tendon reflexes, dysarthria, visual dysfunction and hearing loss [176 - 179]. In addition, non-neurological manifestations including cardiomyopathy, diabetes and skeletal deformities are commonly present [177, 178, 180 -182].

FRDA is the most frequent of the ARCA worldwide with the exception of sub-Saharan Africa and Southeast Asia [183]. It is also the most common ataxia in Caucasians, affecting two – five / 100 000 individuals [13, 184]. The estimated carrier frequency ranges from 1:60 to 1:110 [14, 15]. Ireland is reported to have one of the highest observed FRDA prevalence at 1:23 000 [15]. Typically, the symptom onset in FRDA is between 5 and 20 years of age with mean age at onset of 10.5 ± 7.4 years and 11.6 ± 4.5 years reported by Harding and Filla et al. respectively [180, 185]. Approximately 15% of individuals with FRDA have onset after the age of 25 years and are classified as delayed-onset cases [186, 187].

FRDA is caused by defect in the frataxin (*FXN*) gene on chromosome 9q13-q21.1,57 [92]. The vast majority of affected individuals (96%) are homozygous for a GAA triplet repeat expansion in the first intron of the *FTX* [92], which causes decreased transcription of the mRNA for frataxin to about 10% of normal levels. About 4% of FRDA patients are compound heterozygotes and harbour a GAA expansion on one allele and a point mutation or deletion on the other [92, 188, 189], leading to lack of functional frataxin by abnormal protein folding, premature truncation, and loss of biological activity [10, 92, 178, 188, 190]. Recently, Candayan et al. reported siblings from a consanguineous Turkish family with a Charcot-Marie-Tooth - like (CMT) phenotype and homozygous *FXN* point mutation in the absence of an expanded allele [191].

The majority of expanded alleles associated with FRDA contain between 600 and 1200 GAA repeats [92, 178, 192, 193] with disease causing GAA repeat expansions ranging from 66 to 1300. The shortest repeat length associated with disease has not been clearly determined and an affected individual with a 56 GAA repeat allele has been reported [194]. Thus, the exact differentiation between normal and full-penetrance alleles has not been yet established, and the upper limit of the normal reference range is less definitive. Prior studies have shown that the length of the expanded repeats and particularly the length of the shorter allele (GAA1) inversely correlates with age at disease onset, disease severity and rate of progression [178; 195 - 197].

All genetic abnormalities in the *FXN* gene lead to significantly decreased level of the mitochondrial protein frataxin. Insufficient frataxin results in mitochondrial dysfunction, manifesting as decreased production of adenosine triphosphate (ATP), impaired iron-sulfur cluster assembly, abnormal iron accumulation, generation of reactive oxygen species, increased oxidative stress, and ultimate cell damage [93, 94, 198 - 201]. While FRDA patients have marked reduction in frataxin, individuals carrying one allele of

abnormal *FXN* with approximately 50% of frataxin levels, remain asymptomatic [202], hence increasing frataxin levels to that of carriers is an attractive therapeutic strategy [203].

5.1.2 Ophthalmological findings in FRDA

Although visual symptoms are not always recognised in FRDA, both afferent and efferent visual systems are involved. Until relatively recently, ocular motor abnormalities were the best-characterised signs of the visual system damage in patients with FRDA, reflecting disruption of brainstem-cerebellar circuits [95, 204]. The most common feature is fixation instability, interrupted by involuntary saccades, or square-wave jerks (SWJ), reported by Furman et al. and Fahey et al. in all patients, and by Schols et al. in over a third of affected individuals [95, 205; 206]. Nystagmus is less common but still frequent, present variably in 20 to 60% of cases [95, 178, 180]. Other abnormalities observed in individuals with FRDA include: ocular flutter, saccadic dysmetria with both hypo- and hypermetria, disrupted pursuits [180, 205, 207], and in 20% of patients symptomatic oscillopsia has been reported [95]. Prior studies have documented impaired vestibulo-ocular reflexes (VOR) and compromised visual – vestibular interactions as measured by oculography [95, 208, 209], and abnormal caloric testing in majority of patients [205, 207, 210].

Decreased visual acuity is less commonly reported and has been recorded in approximately 20% of patients [97, 178, 180]. A small proportion of affected individuals may develop rapid bilateral visual loss, mimicking Leber’s hereditary optic atrophy (LHON) [211].

Early studies of the afferent visual system in FRDA were primarily descriptive in nature, reporting optic disc pallor noted on clinical examination with optic neuropathy documented in 30% of affected individuals [180]. With advances in ocular imaging techniques, involvement of the afferent visual pathways in FRDA has been further investigated. A handful of studies have drawn attention to the optical coherence tomography (OCT) findings in FRDA cohorts [34, 96 – 98]. These studies described the peripapillary retinal nerve fibre layer (RNFL) changes associated with FRDA and investigated the correlations between RNFL thickness with disease severity and disease duration. Subsequently in 2018, Parkinson et al. reported that FRDA was associated with the greatest degree of RNFL thinning in comparison to a range of other genetically characterised ataxias [91].

Fortuna et al. (26 patients), Dağ et al. (10 patients / 10 eyes) and Seyer et al. (57 patients / 110 eyes) found statistically significant diffuse / average RNFL thickness reduction in comparison to controls [34, 96, 98]. Decreased average peripapillary RNFL thickness was also documented by Noval et al. (23 patients) [97] and a distinctive pattern of RNFL loss predominantly in the superior quadrant was observed in one study which evaluated 29 eyes [34]. Rojas et al. (8 patients) found statistically significant thinning in the average as well as in the sectorial RNFL in all quadrants compared to controls [100].

Macular thickness in FRDA was normal in one study [97], but below the first percentile for age-matched controls in another [34], while significantly reduced foveal thickness was documented by Dağ et al. [98]. Meaningful decrease in the macular, but not in foveal thickness was documented in the small Spanish study [100].

RNFL thickness has been shown to correlate with neurological function and disability as measured with the International Cooperative Ataxia Rating Scale (ICARS) [96 - 98], although not consistently with the Friedreich's Ataxia Rating Scale (FARS)

[34, 97]. One study found correlation between the peripapillary RNFL and SARA score [99], used to quantify disability in 52 FRDA patients [101], demonstrating that as the disease progresses, the RNFL thickness reduces. Inverse correlation between RNFL with neurological disability measured with SARA score was also observed by Rojas et al. [100]. In the UK study, a significant correlation between ADLQ (Activities of Daily Living Questionnaire) score from the modified FARS assessment and RNFL thickness was also observed [101].

Association between the RNFL thickness and the age of onset has been found in several studies [34, 96, 101], while correlation between RNFL and disease duration, reported by Noval et al., Seyer et al., and Thomas-Black et al., was not seen by Fortuna et al. [34, 96, 97, 101]. No relationship between RNFL with age of onset and disease duration was recorded in the small Turkish study [98].

To date, only one study evaluated RNFL changes in FRDA over time. Rojas et al. performed OCT on 8 FRDA patients at baseline and at a 6 month's follow-up interval, and reported macular and parapapillary RNFL thicknesses decline over this period [100].

Altogether these findings indicate that RNFL structural changes have a functional correlate and thus suggest that OCT measurements, and in particular RNFL parameters, may be a useful marker of disease progression in FRDA patients.

5.2 Specific aims

- a. To identify individuals with FRDA and describe the associated phenotype.
- b. To characterise the retinal findings and measure the peripapillary RNFL and macular thickness with OCT in patients with FRDA, and to compare OCT data to age – and sex-matched healthy volunteers, who will serve as controls.
- c. To determine whether the pattern of retinal changes (when present) in patients with FRDA possesses any specificity.
- d. To determine if correlation can be found between OCT findings as an anatomical marker in individuals with FRDA and disease severity as quantified with SARA score.
- e. To determine if correlation can be found between OCT findings as an anatomical marker in individuals with FRDA and disease duration.
- f. To document if retinal changes detected by OCT are evident over time and thus determine the usefulness of OCT as an imaging biomarker of disease progression in patients with FRDA who have had previous OCT assessment.

5.3 Subjects and methods

5.3.1 Subjects

Adult patients were recruited from the Irish National Ataxia Clinic (NAC) in Tallaght University Hospital. 48 patients with a confirmed diagnosis of FRDA were invited to participate. 48 healthy controls of comparable age and sex and without evidence of either optic disc or retinal disease were also recruited.

5.3.2 Genetic diagnosis

All affected individuals had confirmed molecular diagnosis of FRDA through commercially available testing. Majority of patients had two expanded GAA alleles, while one individual was compound heterozygote for GAA repeat expansion and a point mutation [189].

5.3.3 Clinical assessment

All affected individuals had clinical assessment using a standardised approach, comprising demographic information, history, pedigree, and detailed neurological examination as outlined in Chapter 4.

Scale for the Assessment and Rating of Ataxia

The scale for the Assessment and Rating of Ataxia (SARA, range from 0-40 points with higher scores indicating more severe disease) [99] (Appendix 3) was obtained for each affected individual.

Visual acuity

The best-corrected visual acuity (BCVA) was measured using Snellen chart and results were expressed in logarithm of the minimal angle of resolution (LogMAR) for analysis.

Optical coherence tomography

Each participant had a spectral-domain OCT (SD-OCT) examination with Topcon 3D OCT-2000 as described in detail in Chapter 4.

Follow-up assessment

Follow-up assessment was performed in 20 individuals.

5.3.4 Statistical analysis

Statistical analysis was performed using Microsoft Excel (2013) and IBM® SPSS® Statistics for Windows version 25.0.

A nonparametric Mann-Whitney U test was used to compare demographic differences between patients and controls. For group quantitative analysis a one-way ANOVA was used followed by a post-hoc analysis. RNFL parameters and foveal thickness were correlated with the SARA score and visual acuity using Pearson's correlation test, while the relationship between the OCT parameters and disease duration was analysed using Spearman's rank correlation test.

Changes in RNFL thickness from baseline were assessed for 20 patients who had at least one follow-up visit using Wilcoxon-signed rank test, accounting for expected minimal age-related RNFL thinning [61].

A p value <0.05 was considered statistically significant in all analyses.

5.4 Results

5.4.1 Cohort description

48 Caucasian symptomatic individuals from 42 unrelated families, all of Irish descent, were included. There was a slight predominance of males (n=26, 54.2%).

In all affected individuals presenting symptom was ataxia or gait disturbance. The age-at-symptom-onset ranged between 4 - 40 years (mean 13.8 ± 8.1), while disease duration varied between 5 - 43 years (mean 19.5 ± 9.9). Mean age at assessment was 33.4 ± 13 years (range 18 – 63). Twenty six individuals (54.2%) had no relevant family history. The remaining 22 cases (43.8%) were familial with inheritance compatible with an autosomal recessive mode in all but one family (patient 18, Table 5.1), where a pseudo-dominant pattern of inheritance was observed. In this family disease transmission from one generation to the next was registered with affected mother and a number of siblings, while the father was a carrier.

The demographic and clinical data of FRDA patients are shown in Table 5.1.

Table 5.1 Demographic and clinical characteristics of individuals with FRDA

Pt	Fam Hx	Sex	Age at onset	Age at assessment	Disease duration	SARA	Ataxia	WC bound	Hearing loss	LVH	Diabetes mellitus	Scoliosis / surgery
1	No	M	5	31	26	37	+	+	+	+	+	+ / no surgery
2	No	F	16	19	3	8.5	+	-	-	-	-	-
3	No	M	37	42	5	4.5	+	-	-	-	-	-
4	Yes	F	11	26	15	23	+	+	-	+	-	+ / surgery
5	Yes	M	11	25	14	27.5	+	+	-	+	-	-
6	Yes	M	19	40	21	19	+	+	-	-	-	+ / no surgery
7	Yes	F	19	30	11	11	+	-	-	-	-	-
8	Yes	F	16	30	14	13	+	-	-	-	-	+ / no surgery
9	No	F	16	52	36	36	+	+	-	-	+	+ / surgery
10	No	M	35	47	12	16	+	-	-	-	-	-
11	No	M	11	33	22	38	+	+	+	+	-	+ / no surgery
12	Yes	M	11	41	30	34	+	+	-	+	+	+ / no surgery
13	No	F	11	18	7	18	+	+	-	-	-	+ / +
14	No	F	9	35	26	37	+	+	-	+	-	-
15	No	M	5	21	16	16.5	+	+	-	-	+	+ / +
16	Yes	M	30	63	33	15	+	+	+	-	+	-
17	No	M	3	27	24	25	+	+	-	+	-	+ / +
18	Yes	F	18	54	36	15.5	+	+	-	-	-	-
19	Yes	M	5	18	13	28.5	+	+	-	-	-	+ / -
20	Yes	F	13	19	6	11.5	+	-	-	+	-	+ / -
21	Yes	F	10	24	14	20.5	+	+	-	-	-	+ / +
22	Yes	F	11	27	16	19.5	+	+	-	-	-	+ / +
23	No	M	16	35	19	21	+	+	-	-	-	+ / -
24	No	F	40	60	20	29	+	+	-	-	-	-
25	No	F	5	18	13	24.5	+	+	-	+	+	-
26	Yes	M	14	54	40	27.5	+	+	+	-	+	-
27	Yes	M	23	45	22	28	+	+	-	-	-	+ / -
28	No	F	4	31	27	26	+	+	-	+	+	+ / +
29	Yes	F	12	36	24	30	+	+	+	+	-	-
30	No	M	12	51	39	37.5	+	+	-	+	-	+ / +
31	No	M	10	20	10	23	+	+	-	+	-	+ / -
32	Yes	M	17	47	30	31.5	+	+	-	-	+	+ / -
33	Yes	M	21	45	24	18	+	-	-	-	-	-
34	No	M	13	18	5	11.5	+	-	-	+	-	+ / -
35	No	F	7	21	14	27	+	+	-	-	+	+ / -
36	Yes	M	6	22	16	20.5	+	+	-	+	-	+ / -
37	Yes	F	8	26	18	26	+	+	-	+	-	-
38	No	M	8	18	10	29.5	+	+	-	+	-	+ / -
39	Yes	F	16	51	35	16	+	+	-	-	-	-
40	No	F	10	23	13	19	+	+	-	+	-	+ / -
41	No	M	15	36	21	14	+	-	-	-	-	-
42	No	M	10	19	9	18	+	+	-	+	-	+ / +
43	Yes	F	9	34	25	23.5	+	+	-	-	-	+ / +
44	No	F	11	29	18	22	+	+	-	+	-	+ / -
45	Yes	M	10	53	43	24	+	+	-	-	-	-
46	No	F	10	21	11	10.5	+	-	-	-	-	+ / -
47	No	M	6	22	16	10.5	+	-	+	+	-	+ / -
48	Yes	M	29	44	15	9.5	+	-	-	-	-	-

Fam Hx Family history; LVH Left ventricular hypertrophy; WC wheelchair; SARA Scale for the Assessment and Rating of Ataxia

All patients displayed variable degree of ataxia and majority (36/48, 75%) required a wheelchair for mobilising with usage ranging from occasional to all the time. Cardiomyopathy in its hypertrophic form with concentric thickening of the left ventricle was documented with an echocardiogram in 21/48 (43.8%), while 10 individuals (20.8%) had diabetes. A small proportion of FRDA patients had hearing loss (6/48 (12.5%)), while of the 18 affected with scoliosis individuals 10 had a prior surgery (37.5%).

SARA score was highly variable in this cohort and ranged between 4.5 - 38/40 (mean $21.9/40 \pm 8.5$).

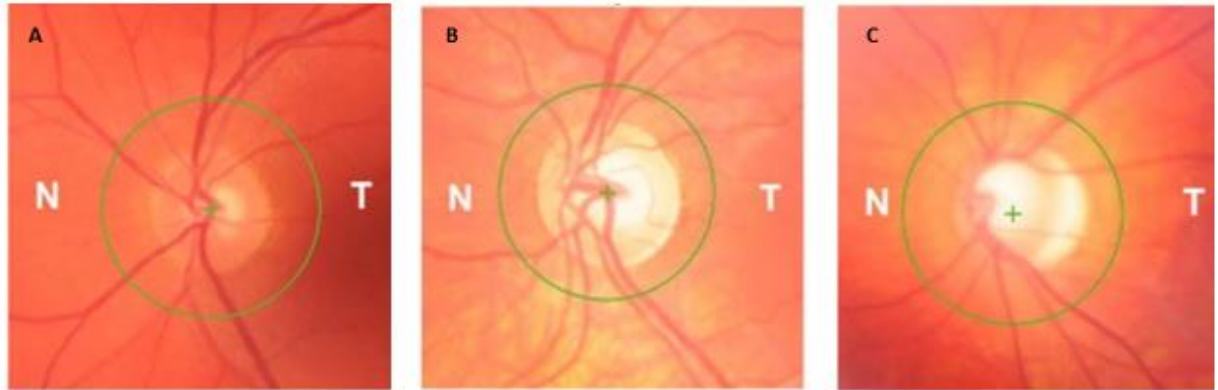
5.4.2 Ophthalmological findings

The mean BCVA in FRDA patients was significantly reduced compared to controls ($p < 0.05$); mean LogMar in patient group was 0.26 ± 0.5 (range 0 to 2.3), while in control group was 0.03 ± 0.07 (range -0.1 to 0.3). In 30/48 patients (62.5%) VA was 20/30 or better and Snellen VA ranged between 20/20 - no light perception.

The majority of FRDA patients ($n=32$, 66.7%) had abnormal ocular motor findings (Table 5.2). SWJ, recognised as small saccades away from and back to midline with intersaccadic intervals, were documented in 28/48 (58%), while one individual had ocular flutter, consisting of horizontal back-to-back saccades without intersaccadic intervals. Less than a half of FRDA patients had nystagmus (20/48, 41.7%).

Fundus examination revealed variable optic disc features ranging from essentially normal appearance to diffuse optic disc pallor. In 21/48 (43.8%) of FRDA patients variable degree of optic disc pallor was documented (Figure 5.1).

Figure 5.1 Retinal fundus images with circular volume scan delineation of FRDA patients with variable degree of optic neuropathy



A. Normal appearance of the optic disc (patient 33); B. Optic disc pallor in a patient with normal visual acuity (patient 2); C. Diffuse optic disc pallor in a patient with reduced visual acuity (patient 21).

5.4.3 Optical coherence tomography

OCT data from all 48 FRDA were compared with those of 48 healthy controls (23 males) with mean age at assessment 33.9 ± 10.2 years (range 16 – 63). There was no difference between patients and controls in terms of gender ($p=0.7$) and age ($p=0.5$).

In total 4 out of 94 eyes were excluded from macular analysis due to poor image quality as a result of severe visual loss or fixation instability, while valid RNFL parameters data were obtained from 69 eyes.

The results of OCT studies are summarised in Table 5.2

Table 5.2 Ophthalmological findings and RNFL results in patients with FRDA

Pt	SWJ	Ny	BCVA		Optic disc pallor	Retinal nerve fibre layer						
			R	L		Average macula (µm)	Foveal (µm)	Average (µm)	Superior (µm)	Nasal (µm)	Inferior (µm)	Temporal (µm)
1	+	+	HM	HM	+	234.6	225					
2	+	-	20/20	20/20	+	276.45	249.5	94	110.5	81.5	115	68.5
3	+	+	20/20	20/25	-	286.9	277	114	122	111	136	87
4	+	+	20/40	20/40	+	267	246	80	101.5	63.5	93.5	63
5	+	+	20/40	20/30	-	259.1	277.5	74	93	55	93	57
6	-	-	20/20	20/20	-	275.55	274	94	101.5	80	127.5	67
7	+	-	20/20	20/20	-	272.35	243.5	108	138	86.5	135	74
8	+	-	20/20	20/20	-	276.85	230	98	106	83	121.5	81.5
9	-	-	NLP	20/50	+	252.8	234					
10	+	-	20/40	20/25	-	290.15	283	104	112	110	109	85
11	+	+	20/70	20/70	-	271.6	238					
12	+	+	20/50	20/50	-	265.8	269					
13	-	-	20/20	20/20	+	264.55	243.5	86.5	99	66	117	76
14	+	+	20/200	20/200	+	254.15	225.5	66	75	95	48	48
15	-	+	20/70	20/30	-	234.3	279	55	62	52	56	50
16	-	-	20/20	20/25	-	260.7	223.5	104	132	78	128	79
17	+	-	20/70	20/40	+	266	274.5	69	70	47	67	54
18	-	-	20/20	20/25	-	272.95	220	107	120	99	120.5	84
19	+	+	20/40	20/40	+	290.8	274					
20	-	+	20/20	20/20	-	279.45	268	99.5	119	101.5	107.5	70.5
21	+	+	20/30	20/30	+	254.75	233	81	87.5	58	107	71
22	-	-	20/25	20/20	-	256	243	82.5	80.5	71.5	100	73
23	+	-	20/20	20/20	-	288.7		74	75	69	85	66
24	+	+	20/30	20/40	-	283.75	210	93	110	68.5	103.5	80.5
25	-	-	20/20	20/25	+	263.1	283	97	116	78.5	113.5	79
26	-	+	20/40	20/40	-	261.1						
27	+	-	20/20	20/20	-	288.85	254	94.5	108.5	86.5	119.5	64
28	+	-	20/20	20/20	+	246.95	207	85	97	67	100	74
29	+	+	20/25	20/30	+	265.3	265.5					
30	+	+	CF	CF	-	267.6	290.5	75	85.5	70	97	60.5
31	+	-	20/25	20/20	-	261.65	277					
32	+	-	20/20	20/20	-	255.15	230	79	95	63	98	62
33	-	+	20/20	20/20	-	272.7	233.5	90.5	103.5	75.5	107.5	74.5
34	+	-	20/30	20/30	-	272.8	210	96	108	54	103	55
35	-	-	20/20	20/20	+	274.1	266.5	98.5	110.5	82	116	87
36	+	-	20/70	20/70	+	258.8	271	87	102.5	80	96	45.5
37	+	+	20/30	20/25	+	273.4	275	76	82.5	70.5	93.5	58
38	+	-	20/50	20/30	-	268.35	267					
39	-	-	20/40	20/40	-	291.35	196.5	105.5	132	82	110.5	97
40	+	+	20/20	20/20	+	265	251	86	88.5	75	107	74.5
41	+	-	20/20	20/20	+	278.9	242.5	99	112.5	81	129.5	74.5
42	-	+	20/20	20/20	+	277.45	267.5	83	89.5	71	110.5	61.5
43	-	-	20/20	20/20	+	266.45	261.5	84.5	75	68.5	118.5	76
44	+	-	20/20	20/20	+	270.15	235	88	95	60	117.5	80.5
45	-	-	20/20	20/20	-	259.05	268.5	80.5	104.5	83	82.5	50.5
46	-	-	20/20	20/20	-	255.45	220	94.5	101	77.5	107.5	93
47	+	-	20/40	20/30	+	272.45	253.5	82.5	73	71	117.5	69
48	+	+	20/20	20/25	-	273.5	239	95.5	108	87.5	109.5	76

SWJ Square wave jerks; Ny Nystagmus; HM Hand Movements; CF Count Fingers; NLP No light perception; Presented OCT values for each patient are the mean of measurements from both eyes unless it was only possible to obtain a good quality scan from one eye.

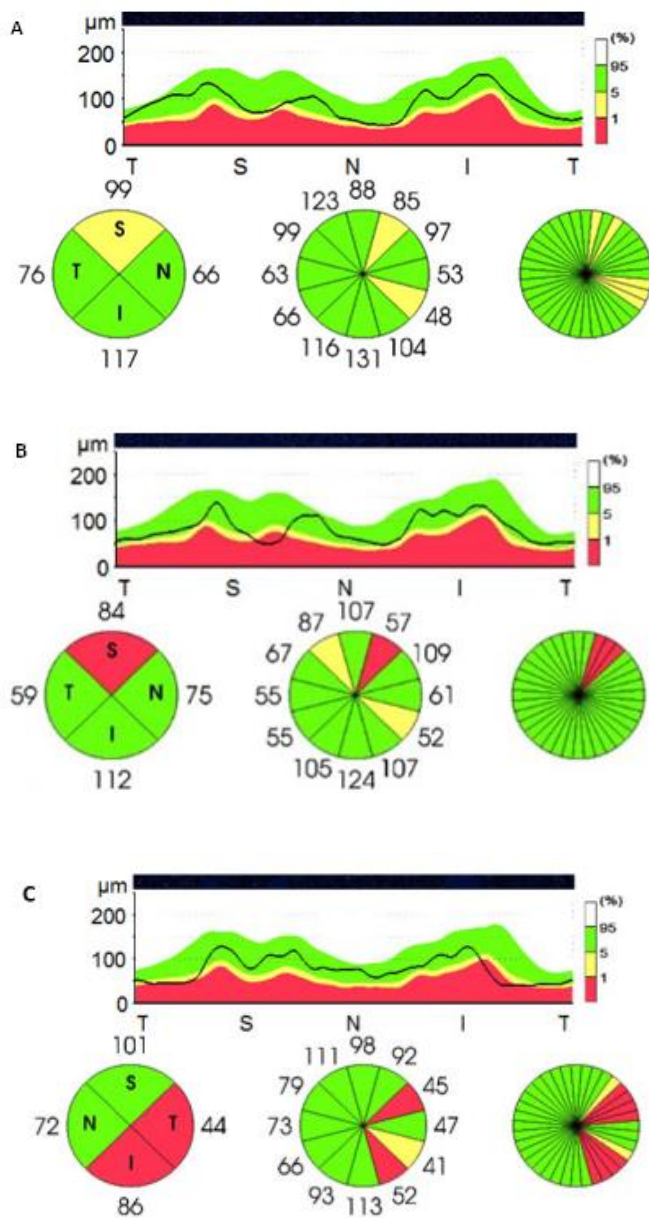
Comparison between FRDA and control groups revealed statistically significant reduction in the average peripapillary RNFL thickness as well as in all sectors except from the temporal in the patient group (Table 5.3). The average macular and foveal thicknesses were also significantly reduced in affected individuals when compared with controls.

Table 5.3 Analysis of OCT measurements between FRDA and control group

					3D Macula		3D Disc				
		M / F ratio	Age (years)	BCVA (LogMAR)	Average (μm)	Foveal (μm)	Average RNFL (μm)	Superior (μm)	Nasal (μm)	Inferior (μm)	Temporal (μm)
FRDA	mean ± SD	26 / 22	33.4 ± 13	0.26 ± 0.5	268.1 ± 13.2	250.4 ± 24.2	88.4 ± 12.9	97.9 ± 20.1	75.4 ± 15.1	105.7 ± 19.4	70.3 ± 12.7
	range		(18 - 63)	(0 to 2.3)	(233.9 - 294.3)	(194 - 300)	(55 - 114)	(58 - 134)	(54 - 111)	(56 - 138)	(51 - 96)
Controls	mean ± SD	23 / 25	34.2 ± 10.2	0.03 ± 0.07	273.8 ± 10.9	238.3 ± 22.8	103.9 ± 8.4	122.3 ± 12.9	88.5 ± 10.1	130.6 ± 11.3	74.5 ± 7.8
	range		(16 - 63)	(-0.1 to +0.3)	(252 - 300)	(214 - 293)	(92 - 124)	(90 - 155)	(72 - 124)	(110 - 156)	(60 - 96)
p value		0.6	0.5	0.00003	0.02	0.01	< 0.00001	< 0.00001	< 0.00001	< 0.00001	0.06

43.6% (17/39) of individuals had superior quadrant thickness below the 95% lower limit of normal, defined in the normative database provided by the manufacturer (Figure 5.2). 28.2% (11/39) had inferior quadrant reduction below the 95% lower limit, while nasal and temporal thickness reduction below the 95% lower limit of normal was documented in 5, and 4 individuals, respectively. The OCT of the individual with a GAA repeat expansion on one allele and a point mutation on the other allele showed significant sectorial RNFL thinning below the normal limits both in temporal and inferior quadrants (Figure 5.2).

Figure 5.2 Optical coherence tomography of patients with FRDA



Pie graphs of quadrants (T = temporal; S = superior; N = nasal; I = inferior) and RNFL circular tomogram representing quantitative analysis of RNFL thickness (black line) and normative data set (green area = 95% confidence interval, yellow area = 99% CI, red area = outside 99% CI). This figure demonstrates reduced sectorial superior quadrant thickness in patients 42 (A), and 5 (B), and reduced temporal and inferior quadrant thickness in patient 45 (C).

5.4.4 Correlation with clinical features

There was significant correlation between baseline macular, average peripapillary and RNFL thickness in all sectors but the nasal and disease severity as quantified with SARA score (Table 5.4). Moreover, analysis revealed that visual acuity was correlated with the average macular and RNFL thickness. There was no significant association found between disease duration and RNFL thickness, but relationship was almost significant for the macular thickness.

Table 5.4 Correlation between OCT parameters and disease severity, disease duration and visual function

	Macula		RNFL				
	Average	Foveal	Average	Superior	Nasal	Inferior	Temporal
SARA	$r(47)=-0.320$	$r(45)=0.080$	$r(38)=-0.56$	$r(38)=-0.42$	$r(38)=-0.308$	$r(38)=-0.520$	$r(38)=-0.399$
	p=0.03	p=0.6	p=0.0002	p=0.008	p=0.06	p=0.0008	p=0.01
Disease	$r_s = -0.286$	$r_s = -0.259$	$r_s = -0.266$	$r_s = -0.165$	$r_s = -0.057$	$r_s = -0.195$	$r_s = -0.137$
duration	p=0.051	p=0.09	p=0.1	p=0.3	p=0.7	p=0.2	p=0.4
BCVA	$r(47)=-0.391$	$r(45)=-0.015$	$r(38)=-0.396$	$r(38)=-0.292$	$r(38)=-0.089$	$r(38)=-0.44$	$r(38)=-0.374$
	p=0.006	p=0.9	p=0.01	p=0.08	p=0.6	p=0.005	p=0.02

Relationships between the OCT parameters and disease duration were analysed using the Spearman's rank correlation test; relationships between the OCT parameters and disease severity as quantified with SARA score, and visual acuity were analysed using Pearson's correlation.

5.4.5 Longitudinal assessment

20 individuals had further OCT studies during a mean follow-up interval of 28.4 months (range 12 - 48). Only macular OCT images from one individual (patient 29) were analysed due to poor image quality at both assessments.

There was significant decline in the average peripapillary RNFL thickness (Figure 5.3) as well as in most of the RNFL sectors over time (Table 5.5; Table 5.6). Statistically significant thinning was also documented in the average macular thickness (Figure 5.4) and the fovea (Figure 5.5). Disability progression, documented in 19/20 patients, is shown in Figure 5.6 (mean SARA at baseline 18.9 ± 6.5 , at follow-up 21.9 ± 6.5 , $p < 0.05$).

Table 5.5 Analysis of macular and RNFL thickness over time

	3D Macula		3D Disc				
	Average (μm)	Foveal (μm)	Average RNFL (μm)	Superior (μm)	Nasal (μm)	Inferior (μm)	Temporal (μm)
Baseline	270.3 ± 12.4	257.9 ± 22.3	88.8 ± 14.3	97.6 ± 20.6	75.8 ± 17.1	105.4 ± 18.9	68.9 ± 12.5
Follow up	267.4 ± 12.4	246.9 ± 24.1	85.2 ± 15.5	92.6 ± 24.4	72.5 ± 14.9	105.1 ± 21.4	67.1 ± 12.2
p value	0.009	0.007	0.007	0.016	0.036	0.046	0.054

Table 5.6 Baseline, follow-up and expected follow-up RNFL thickness in FRDA patients

Patient	Baseline RNFL thickness	Follow up RNFL thickness	Expected follow up RNFL thickness*	Difference	Time from baseline
2	94	93	93.67	0.33	12
3	114	106.5	113.37	6.87	23
4	81	80	80.175	0.18	30
5	74	73	73.175	0.18	30
6	94	93	93.67	0.33	33
7	108	106	107.37	1.37	23
10	104	90.5	103.37	12.87	23
15	55	44	54.2	10.2	29
18	107	103	105.68	2.68	48
21	81	72.5	80.18	7.68	30
23	74	66	73.2	7.18	30
25	97	95.5	96.18	0.68	30
33	90.5	90	90.12	0.12	14
34	96	94	95.56	1.56	16
36	87	85.5	86.15	0.65	31
37	76	67	75.18	8.18	30
42	83	82.5	82.1	-	33
43	84.5	83	83.73	0.73	28
44	88	86.5	87.23	0.73	28

*Minimal thinning reported in healthy subjects (0.33 $\mu\text{m}/\text{year}$)

At follow-up assessment significant correlation between RNFL thickness and disease severity as quantified by SARA score was found once more ($r_{\text{Average RNFL vs SARA}} = -0.467$, $p = 0.04$). Similarly to baseline assessment, there was no association between disease duration and RNFL or macular thickness ($r_{\text{Average RNFL vs duration}} = -0.291$, $p=0.22$; $r_{\text{Macula vs duration}} = -0.059$, $p=0.8$).

Figure 5.3 Average RNFL thickness at baseline and at follow-up evaluation

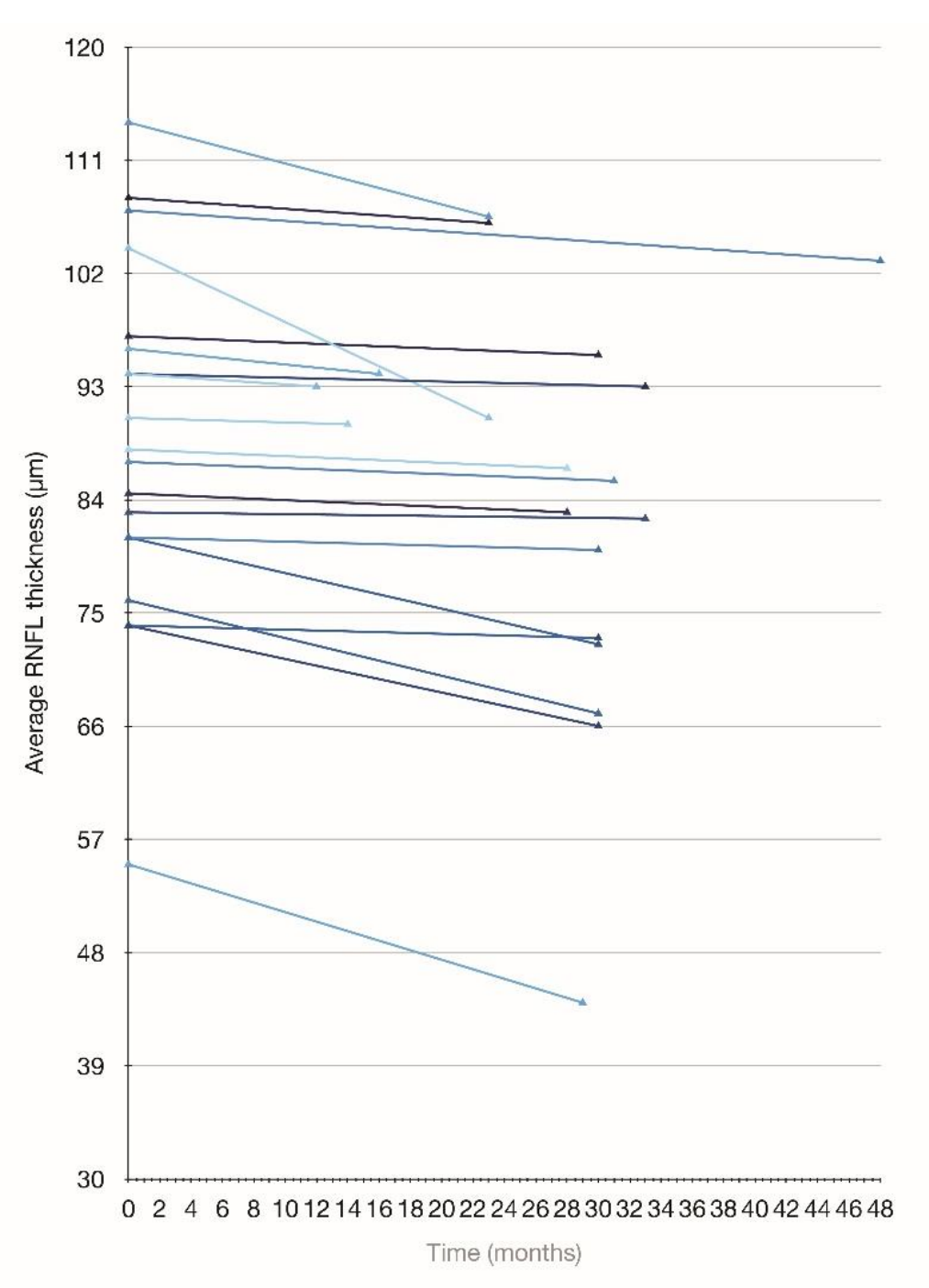


Figure 5.4 Macular thickness at baseline and at follow-up evaluation

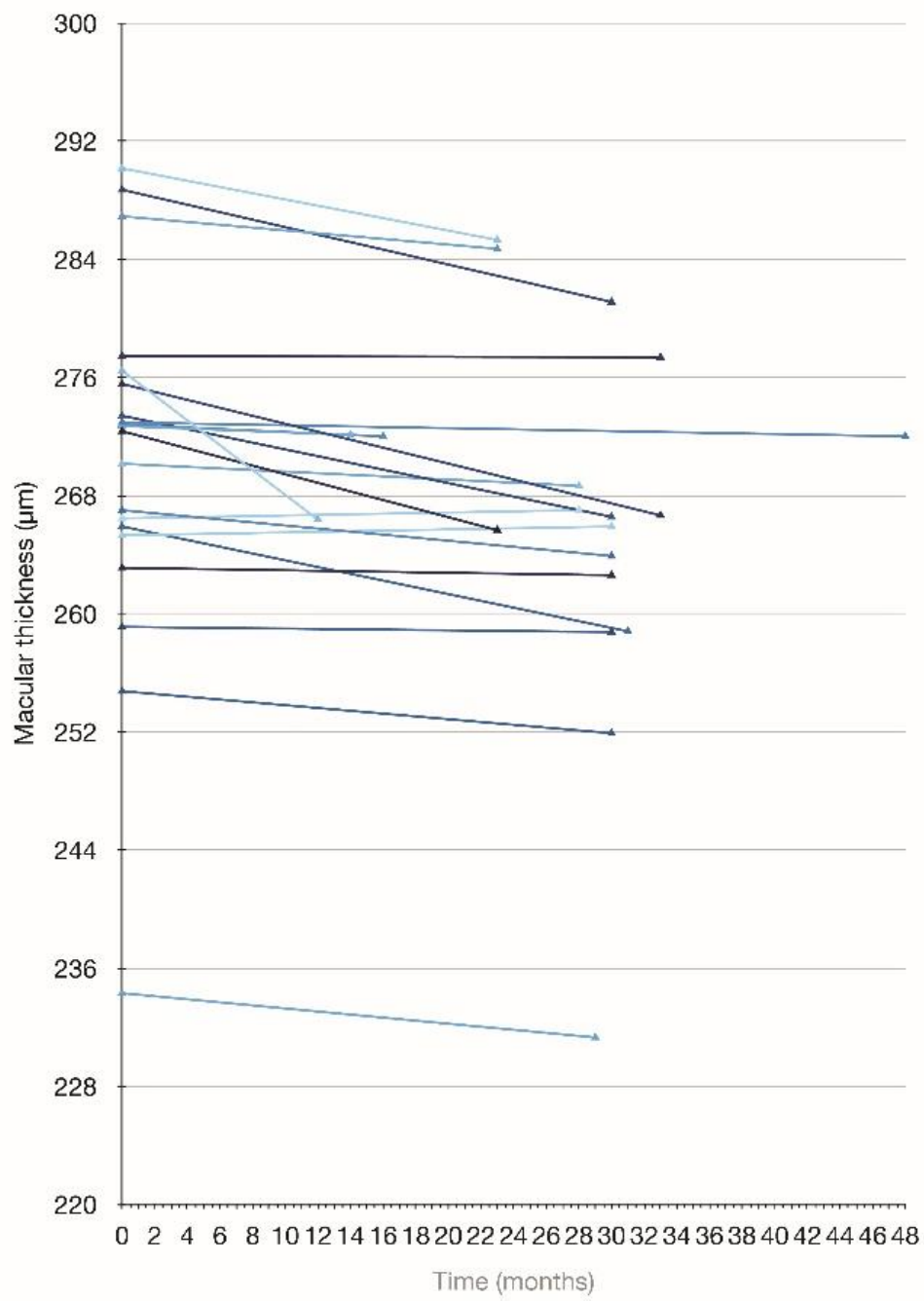


Figure 5.5 Foveal thickness at baseline and at follow-up evaluation

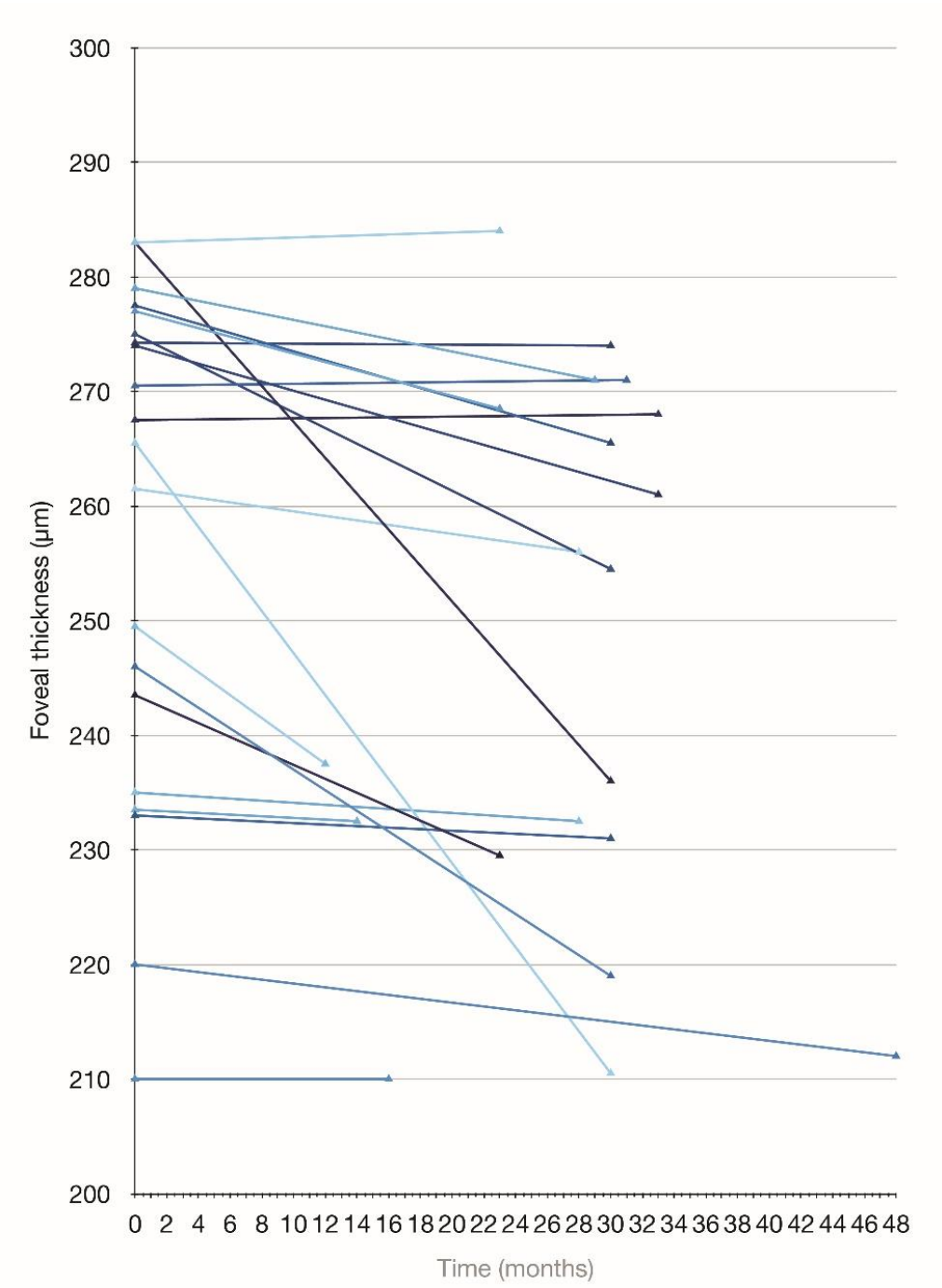
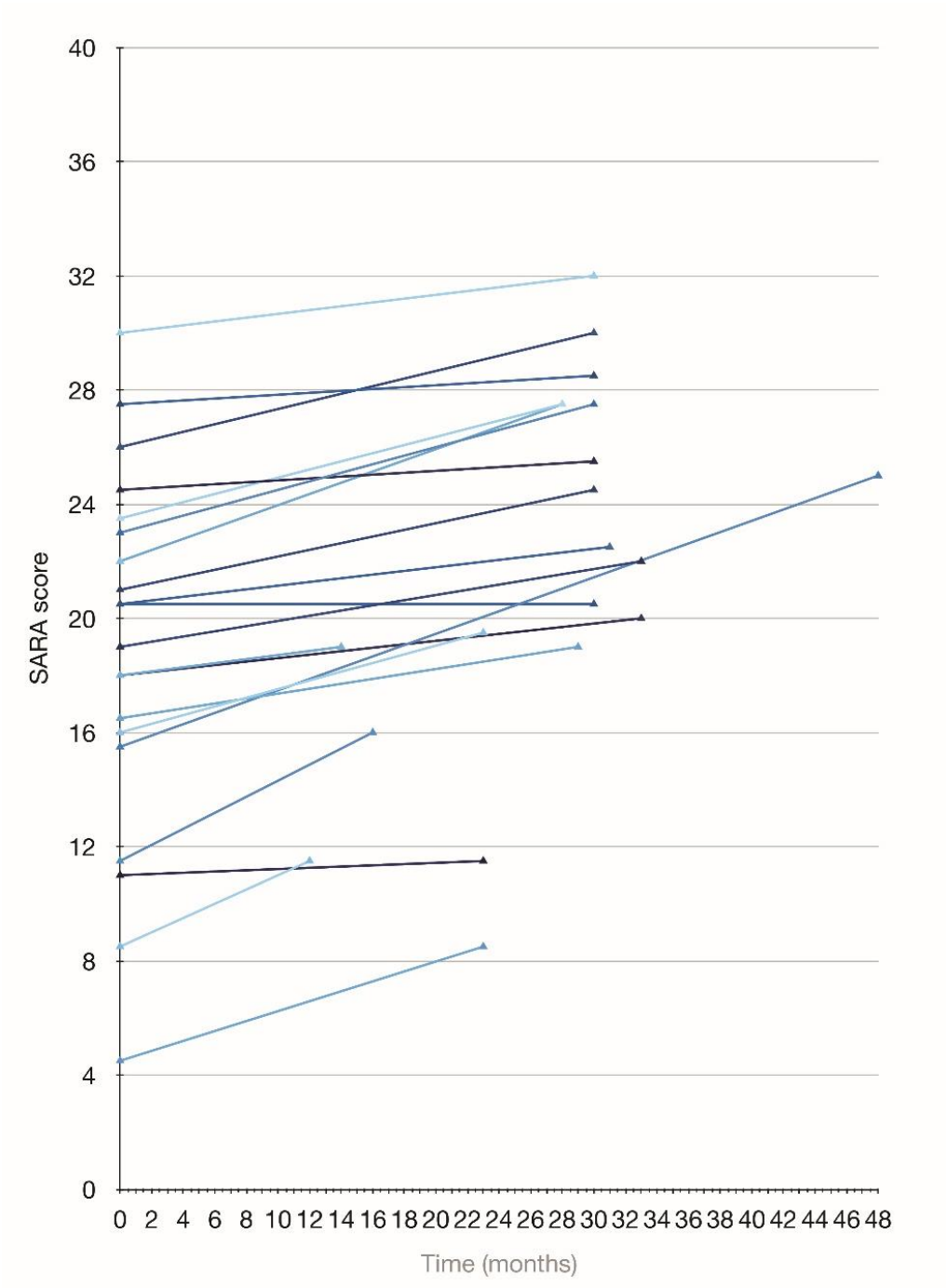


Figure 5.6 SARA score at baseline and at follow-up assessment



5.5 Discussion

This is the first Irish study systemically investigating retinal involvement in individuals with FRDA using OCT. The results of this research confirm significant RNFL and macular thinning in this entity even in patients without clinically apparent visual impairment and highlight the usefulness of OCT to detect RNFL thickness changes with the progression of the disease. A modest number of previous OCT studies evaluated retinal changes in FRDA and our cohort compares to the two largest published patient groups: 57 individuals in the US, and 52 individuals in the UK studies [34, 101]. Similarly to an Italian study in which 80% of the 26 FRDA patients were completely visually asymptomatic, but all had evidence of underlying optic neuropathy detected with an OCT [96], individuals with FRDA were found to exhibit some degree of visual pathway involvement, apparent clinically only in a small proportion of affected subjects.

In this non-homogeneous FRDA cohort both functional (BCVA) and structural (OCT) visual measures correlated with neurological disability as quantified with SARA score, indicating that these can potentially serve as functional and anatomic measures of disease progression in future clinical trials. Similar findings were reported by Seyer et al. [34], where correlations between OCT parameters and neurologic function as measured by FARS were slightly higher than associations with visual functions, proposing that OCT may ultimately be applicable as an anatomic biomarker of structural neuronal loss in FRDA.

The pattern of RNFL loss in FRDA is characteristic. The results of this study confirm significant average parapapillary RNFL thickness reduction, consistent with previous reports [34, 91, 96 - 98, 100, 101]. In contrast to other nuclear mitochondrial disorders where temporal quadrant RNFL is most affected [212], in this FRDA cohort the RNFL

thickness in the temporal region was least decreased, similarly to previously reported observations [97]. Moreover, close to half of our patients had superior quadrant involvement, reported as the predominantly affected sector in FRDA by Seyer et al. [34]. Notably, when compared with individuals with other ataxia subtypes evaluated in this research (Chapters 6, 7, 8, 9, 10, and 11), the peripapillary RNFL thickness in the FRDA cohort was significantly thinner than in the other groups (each $p < 0.05$). However, no statistically significant difference was found in comparison to genetically undetermined ataxia group. The OCT results are comparable with one previous study where among determined inherited ataxias, FRDA was associated with the most notable RNFL thinning [91], and thus suggest the potential value of OCT in distinguishing FRDA from other progressive ataxias.

The classical optic neuropathy of mitochondrial dysfunction is associated with marked preference for damage to the papillo-macular bundle, which contains the highest density of the retinal ganglion cells (RGCs) comprising the optic nerve, resulting in central vision loss and temporal pallor of the optic nerve [211, 213]. LHON and dominant optic atrophy (DOA), the most common non-syndromic mitochondrial optic neuropathies, share overlapping clinical and pathological features and are characterised by early loss of RGCs within the papillo-macular bundle [40, 211], leading to characteristic temporal loss. The selective vulnerability of the optic nerve in classical mitochondrial optic neuropathies has been related to uneven distribution of mitochondria and thus unequal energy demands along each RGC axon [214]. Histochemical studies have shown mitochondrial clustering in areas with a high density of repolarising sodium-potassium membrane pumps, and an abrupt decrease in mitochondrial numbers posterior to the lamina cribrosa where myelination begins and energy-efficient saltatory conduction occurs [211, 215, 216]. A combination of energy failure, oxidative stress, and

predisposition to apoptosis along with mitochondrial distribution within this axonal system is considered the pathological basis for RGC degeneration in both LHON and DOA [217].

In contrast to LHON and DOA, optic neuropathy observed in FRDA patients does not follow the classical mitochondrial pattern. The papillo-macular axonal system is not preferentially involved, suggesting that in the mitochondrial disorder associated with genetic defect in *FXN*, the pattern of visual impairment and the underlying pathological mechanism are different from those in LHON and DOA [96]. The encoded mitochondrial protein frataxin is directed to the mitochondrial inner membrane and is involved in the assembly of iron–sulphur clusters, which are critical components of the mitochondrial respiratory chain complexes [218]. Alterations in frataxin affect the respiratory chain and lead to bioenergetic impairment, increased oxidative stress, and abnormal accumulation of intra-mitochondrial iron, which eventually reaches toxic levels [94], thus increasing sensitivity of cells to undergo apoptosis [93, 94, 198, 200, 201, 219]. Alldredge et al. set the hypothesis regarding the mechanism by which decreased expression of frataxin could cause pathologic changes in restricted groups of tissues and proposed that optic neuropathy in FRDA is a result of the increased sensitivity of RGC to oxidative stress. These cells are relatively resistant to peroxide – induced oxidative stress, but are highly redox sensitive. It is likely that an impaired cellular defence against reactive oxygen species in FRDA further exacerbates neuronal loss [220]. As the cells have some mechanisms for managing reactive oxygen species, the cumulative damage in the retina occurs gradually, leading to visual impairment [221].

By definition, the non-syndromic optic neuropathies are limited to a single cellular target, i.e. the RGCs. These seem more specifically related to a defective complex I function [211] and involve a more specific subset of visual fibres. In contrast, in FRDA complexes I, II and III are involved which may be limiting the compensatory mechanisms likely existing in LHON and DOA, and thus causing less selective damage to RGC populations [96]. Furthermore, the clinical expression in FRDA is associated with much more severe and widespread presentation, behaving as multi-systemic mitochondrial disorder also affecting the optic nerve. Interestingly, despite different pathological mechanisms involving the visual pathways in FRDA, a subacute or acute visual failure mimicking the LHON may develop in the presence of high GAA triplet expansion or in compound heterozygotes, and overlap the slow progression of FRDA optic neuropathy [96, 222]. In contrast to previously reported higher incidence of optic disk pallor in compound heterozygotes than in expansion homozygotes [190], our single compound heterozygous individual was visually asymptomatic and had no disc pallor on fundoscopy.

Only a handful of studies have evaluated the macular thickness in FRDA patients. Similarly to the US and the small Spanish groups [34, 100], macular thickness in this FRDA cohort was reduced compared to controls, in contrast to the larger Spanish study, where macular thickness was normal, but no comparison to controls was documented [97]. Furthermore, in this FRDA cohort significantly reduced foveal thickness was found, previously only documented in 10 eyes of 10 Turkish patients [98], but not observed by Noval et al., and Rojas et al. [97, 100], and not reported in other studies. Previous research which focused on electroretinography did not detect substantial retinal abnormalities in FRDA and it was felt that the loss of low-contrast vision and peripheral visual field with preservation of high-contrast vision was against a primary macular

disease [223]. However, more recent OCT data suggest that macular changes may be a component of the most advanced FRDA visual impairment, and could clinically impair vision only in the most severely affected patients [34].

Similarly to two other studies, a correlation between RNFL thickness and disability as measured by SARA score was found in this cohort, showing that as the disease progresses and neurological function deteriorates, the RNFL decreases [100, 101]. In prior studies, RNFL thickness was correlated to disability as measured with two other much more extensive ataxia rating scales: ICARS and FARS [226, 227]. While no association was found between RNFL thickness and disease severity as measured by FARS, in a study of 23 FRDA patients [97] a correlation was found between RNFL thickness and ICARS, the only scale that considers features not directly related to the physical examination, such as activities of daily living. Three more studies documented correlations between RNFL and disability as quantified with ICARS [96, 97] and FARS [34]. Overall, these findings suggest that RNFL thickness might be a useful marker of disease progression in FRDA and that OCT may have an advantageous role in therapeutic trials.

In this study no correlation was found between RNFL thickness and disease duration, observed in some FRDA cohorts [34, 99, 101], but not documented in others [96, 97]. This discrepancy is possibly due to the highly variable disease duration in this FRDA group in comparison to other cohorts where association was found, although here the number of included subjects was a lot higher than in the two cohorts where no correlation was found. Findings from a small number of OCT studies in various SCAs have suggested that RNFL changes are unrelated to disease duration, similarly to our results [105, 109, 110].

To my knowledge this is only the second longitudinal OCT study in FRDA patients including 2.5 times more subjects than in the Spanish cohort of 8 patients [100]. The results demonstrate significant retinal decline and disability progression over time, confirming prior observations on FRDA patients evaluated at a much shorter time interval of 6 months. The interpretation of results in longitudinal OCT studies may be challenging due to the insufficient knowledge regarding the rate of RNFL loss in this entity. Henderson et al. speculated that RNFL thinning in MS, another degenerative disease of the CNS, is most probably not a linear process and that there is a more rapid RNFL loss in the early stages of the disease [228]. In a small longitudinal study of four individuals with SCA3, 5 out of 8 eyes evaluated showed mild trend to RNFL thinning and a progression rate per year was calculated [109]. However, because of the small sample size and the absence of a control group, it is unclear if the detected loss represented progression of the disease or was attributable to other causes such as variability between exams. In this study, healthy controls were not evaluated at an interval, and thus no comparisons were made. To date, it remains uncertain if there is any dynamic of RNFL thinning during the FRDA disease course. It would be interesting to see if the significant progressive reduction of RNFL, observed in this study, can be replicated in FRDA longitudinal studies where different generation OCT devices are used.

Two of previously reported OCT studies in FRDA were performed entirely using SD-OCT [98, 100]. One of the groups used TD-OCT for RNFL evaluation and SD-OCT for the macular analysis [34], while TD-OCT was utilised in the remaining FRDA studies [96, 97, 101]. Prior evaluation of the intersystem reproducibility showed that there is discrepancy between results from different OCT devices. In a cross-sectional study of 52 healthy eyes, the macular thickness measurements obtained by SD-OCT devices were

higher than those obtained by TD-OCT [224]. Furthermore, RNFL analysis of patients with MS and healthy controls performed with TD- and SD-OCT found a strong correlation, but a statistically significant difference between the two devices, suggesting that measurements between different generations of OCT machines (TD-OCT versus SD-OCT) are not interchangeable. The discrepancy between the measurements obtained with different devices may have particular implications for longitudinal studies if switching OCT machine when monitoring retinal changes over time [225].

A number of potential therapeutics have been tested in clinical trials in FRDA patients, but at present there is no approved therapy. Furthermore, the disease typically has a long advancement period, making disease progression studies problematic. In a number of other neurodegenerative diseases the need for disease-modifying treatments has been facilitated by identification of potential markers that can capture subclinical changes in a rapid manner, and thus can be useful in clinical trials in slowly progressive neurological disorders. In FRDA, important progress has been made in addressing the various aspects of biomarkers use, including their diagnostic, monitoring, response, predictive, and prognostic role [148]. In contrast to previously proposed FRDA biomarkers, OCT is a non-invasive, quick and increasingly available test for identifying characteristic changes in individuals with FRDA, which is easy to perform. Moreover, this technique demonstrates high reproducibility and thus, has the potential to be reliably utilised in future clinical trials.

5.6 Conclusions

This study highlights that FDRA is associated with frequent subclinical optic neuropathy. The results demonstrate that RNFL thickness as measured by OCT has the potential to become a quantifiable biomarker for the evaluation of disease progression. Furthermore, the longitudinal data showing significant progression rates of retinal damage detectable through OCT support the usefulness of this cost-effective technique as objective tool in future therapeutic trials.

Chapter 6: Clinical phenotype and optical coherence tomography in

SPG7 - related spastic ataxia

6.1 Introduction

6.1.1 Hereditary spastic paraplegias and *SPG7*- associated spastic ataxia

The hereditary cerebellar ataxias (CA) and hereditary spastic paraplegias (HSP) comprise diverse group of clinically and genetically heterogeneous disorders. While HSP, characterised by a length-dependent distal axonopathy of the corticospinal motor neurons, leads to lower limb spasticity and weakness in pure forms, CA classically presents with progressive cerebellar syndrome resulting from cerebellar degeneration. Furthermore, a large variety of additional neurological and non-neurological signs may complicate the phenotype in both entities.

As discussed in more detail in Chapter 1, there is significant genetic and phenotypic overlap between CA and HSP.

Spastic Paraplegia type 7 gene (*SPG7*, OMIM#607259) encoding paraplegin, originally identified as a cause of autosomalrecessive (AR) HSP [229], was subsequently recognised as one of the most common ARCA worldwide [16, 161]. It is the second most common diagnosis in the NAC [5]. *SPG7*-associated disease is often complex with relatively rarely described specific features and genotype-phenotype correlations [160, 161, 229].

6.1.2 Ophthalmological findings of *SPG7* - related spastic ataxia

A diverse range of ocular motor abnormalities, including ptosis and progressive external ophthalmoplegia [230] have been reported in *SPG7* cases. Optic nerve involvement, common in neurodegenerative mitochondrial disorders, has also been described in complex HSP [40, 130], including *SPG7*-associated spastic ataxia [130, 131, 140]. Paraplegin-associated optic neuropathy, recognised as disc pallor on ocular fundus examination, has been documented in almost 10% of published *SPG7* cases [161] and rarely reported as a presenting feature [11, 160]. Small case series (3 patients in Wiethoff et al. and 10 patients in Klebe et al. studies) have reported subclinical optic neuropathy even in patients with little or no evidence of visual dysfunction, detected as an abnormal thinning of the retinal nerve fibre layer (RNFL) to a degree measurable by OCT [130, 131]. More recently reported cohort of 23 highly heterogeneous genetically HSP patients [132] demonstrated thinning of the RNFL, which was mostly mild-to-moderate and was not a constant feature of complex HSP, but was more widespread and extended to the pure forms. RNFL changes did not affect any specific quadrants, but notably spared the temporal area. However, the sample size of only 2 individuals with *SPG7*-associated spastic ataxia was very small for meaningful conclusion. Observed RNFL thinning correlated with age and disease duration, but not with clinical severity as quantified with the Spastic Paraplegia Rating Scale (SPRS).

No larger studies have been performed to characterise further if RNFL findings in *SPG7* cohorts possess any specificity or to investigate relationship of retinal findings with disease severity. Progressive RNFL thickness changes have been demonstrated in other neurodegenerative disorders [81], but little is known about retinal changes over time in the *SPG7* cohort.

6.2 Specific aims

- a. To identify individuals with biallelic recessive *SPG7* variants and describe the associated phenotype.
- b. To characterise the retinal findings and measure the peripapillary RNFL and macular thickness with OCT in patients with recessive *SPG7*-associated spastic ataxia, and to compare OCT data to age – and sex-matched healthy volunteers, who will serve as controls.
- c. To determine whether the pattern of retinal changes (when present) in patients with *SPG7*-associated spastic ataxia possesses any specificity.
- d. To determine if correlation can be found between OCT findings as an anatomical marker in individuals with *SPG7*-associated spastic ataxia and disease severity as quantified with SARA score.
- e. To determine if correlation can be found between OCT findings as an anatomical marker in individuals with *SPG7*-associated spastic ataxia and disease duration.
- f. To document if retinal changes detected by OCT are evident over time, and thus, determine the usefulness of OCT as an imaging biomarker of disease progression in patients with *SPG7*-associated spastic ataxia who have had previous OCT assessment.

6.2.1 Secondary objectives

To perform colour vision testing on affected individuals; to compare OCT RNFL measurements of individuals with *SPG7*-associated spastic ataxia with OCT findings in heterozygous *SPG7* carriers; to determine if correlation can be found between RNFL

thickness and visual function in affected individuals with *SPG7*–associated spastic ataxia; and to characterise neurophysiological and neuroimaging findings in this cohort.

6.3 Subjects and methods

6.3.1 Subjects

Adult patients were recruited from the Irish National Ataxia Clinic in Tallaght University Hospital with further three recruited from the Dublin Neurological Institute at the Mater Misericordiae University Hospital. Thirty-one symptomatic patients with a molecular diagnosis of *SPG7*-associated disease and one symptomatic individual with suspected *SPG7* were invited to participate. Thirty-two healthy controls of comparable age and sex, 7 individuals heterozygous for *SPG7* mutation (4 asymptomatic first-degree relatives of affected patients and 3 unrelated individuals with progressive, to date genetically undetermined ataxia), and 1 asymptomatic individual, compound heterozygous for two *SPG7* variants were also recruited.

All patients underwent extensive investigations, including laboratory, genetic and other tests to exclude an alternative diagnosis.

Nerve conduction studies (NCS) were carried out in Neurophysiology Department of TUH with the Dantec® Keypoint® Focus System (Keypoint.Net 2.32) and involved sampling of upper and lower limb sensory and motor nerves.

Routine clinical 1.5-T magnetic resonance imaging (MRI) brain of all patients who underwent MRI were reported by a radiologist experienced in neuroradiology and, when images were available, were also reviewed at the NAC MDT meeting. Axial fluid-attenuated inversion recovery (FLAIR), axial T2-weighted and sagittal T1- and/or T2-sequences were used to evaluate cerebellar atrophy, which was graded by consensus as mild, moderate or severe in each case.

6.3.2 Genetic diagnosis

Molecular diagnosis in most affected individuals was obtained through commercially available next-generation sequencing (NGS) gene panel testing. In all remaining participants with a known familial diagnosis, the relevant *SPG7* variants were tested directly. Of the three individuals with single heterozygous *SPG7* variants identified by targeted NGS gene panels, one was subsequently found to have intronic repeat expansions in the *RFC1* gene, thus confirming a diagnosis of cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) [171]. The remaining two did not have copy number variations with *SPG7* MLPA (Multiplex ligation-dependent probe amplification), both were also negative for the common repeat expansions and had negative whole-exome sequencing (WES).

6.3.3 Clinical assessment

All affected individuals had clinical assessment using a standardised approach, comprising demographic information, history, pedigree, and detailed neurological examination as outlined in Chapter 4.

Scale for the Assessment and Rating of Ataxia

The Scale for the Assessment and Rating of Ataxia (SARA, range from 0-40 points with higher scores indicating more severe disease) [99] (Appendix 3) was obtained for each affected individual.

Visual acuity and colour vision

The best-corrected visual acuity (VA) was measured using Snellen chart and results were expressed in logarithm of the minimal angle of resolution (LogMAR) for analysis. Colour vision was tested using Ishihara test plate [173].

Optical coherence tomography

Each participant had a spectral-domain OCT (SD-OCT) examination with Topcon 3D OCT-2000 as described in details in Chapter 4.

Follow-up assessment

Follow-up assessment was performed in 14 individuals.

6.3.4 Statistical analysis

Statistical analysis was performed using Microsoft Excel (2013) and IBM® SPSS® Statistics for Windows version 25.0.

A nonparametric Mann-Whitney U test was used to compare demographic differences between patients and controls. For group quantitative analysis a one-way ANOVA was used followed by a post-hoc analysis. Following Shapiro-Wilk's test for normality, Kruskal–Wallis test was performed for comparison between the smaller groups. RNFL thickness and foveal thickness were correlated with the SARA score and disease duration using Pearson's correlation test.

Changes in RNFL thickness from baseline were assessed for 14 patients who had at least one follow-up visit using Wilcoxon-signed rank test, accounting for expected minimal age-related RNFL thinning [61].

A p value <0.05 was considered statistically significant in all analyses.

6.4 Results

6.4.1 Cohort description

Thirty-two Caucasian symptomatic individuals with biallelic *SPG7* variants from 25 unrelated families, all of Irish descent, were included (Table 6.1). The majority were male (n=23, 71.9%). There was no difference between affected individuals and healthy controls in terms of gender (p=0.3) and age (p=0.6).

In all affected individuals presenting symptom was ataxia or gait disturbance. The age-at-symptom-onset ranged between 12 and 61 years (mean 39.1 ± 12.8), while disease duration varied between 5 and 45 years (mean 17.8 ± 9.8). Eighteen individuals (56.3%) had no relevant family history, the remaining 14 cases (43.7%) were familial with inheritance compatible with an autosomalrecessive mode. There were no families with disease transmission from one generation to the next.

Detailed clinical characteristics are shown in Table 6.2.

Table 6.1 Individuals with *SPG7*-associated spastic ataxia

Patient	Family	Sex	Age at onset	Age at assessment	Disease duration	Nucleotide change	Amino Acid change	Predicted effect
1	A	M	51	62	11	Hom c.1529C>T	Hom p.Ala510Val	Missense
2	B	M	50	69	19	c.1053dup c.1045G>A	p.Gly352fs p.Gly349Ser	Frame shift Missense
3	C	M	48	67	19	Hom c.1529C>T	Hom p.Ala510Val	Missense
4	D	M	15	48	33	c.1529C>T c.1053dupC	p.Ala510Val p.Gly352fs	Missense Frame shift
5	E	M	42	49	7	Hom c.1529C>T	Hom p.Ala510Val	Missense
6	F	M	12	53	41	c.1529C>T c.850_851delinsC	p.Ala510Val p.Phe284fs	Missense Frame shift
7	G	M	32	44	12	Hom c.1529C>T	Hom p.Ala510Val	Missense
8	H	F	28	66	38	Hom c.1529C>T	Hom p.Ala510Val	Missense
9	H	F	40	64	24	Hom c.1529C>T	Hom p.Ala510Val	Missense
10	I	M	51	74	23	c.1192C>T c.2246C>T	p.Arg398* p.Pro749Leu	Nonsense Missense
11	J	M	55	61	6	Hom c.1529C>T	Hom p.Ala510Val	Missense
12*	K	M	61	67	6	c.1529C>T c.1781T>C	p.Ala510Val p.Val594Ala	Missense Missense
13	L	F	30	54	24	c.1049_1077del c.1715C>T	p.Pro350Glnfs*36 p.Ala572Val	Frame shift Missense
14	L	M	30	45	15	c.1049_1077del c.1715C>T	p.Pro350Glnfs*36 p.Ala572Val	Frame shift Missense
15	L	F	25	43	18	c.1049_1077del c.1715C>T	p.Pro350Glnfs*36 p.Ala572Val	Frame shift Missense
16	M	M	50	62	12	Hom c.1529C>T	Hom p.Ala510Val	Missense

17	N	F	20	39	19	Hom c.1715C>T	Hom p.Ala572Val	Missense
18	O	M	50	68	18	Hom c.1529C>T	Hom p.Ala510Val	Missense
19	O	M	58	69	11	Hom c.1529C>T	Hom p.Ala510Val	Missense
20	O	F	48	59	11	Hom c.1529C>T	Hom p.Ala510Val	Missense
21	O	M	35	52	17	Hom c.1529C>T	Hom p.Ala510Val	Missense
22	P	F	42	55	13	c.1529C>T c.2084T>C	p.Ala510Val p.Leu695Pro	Missense Missense
23	Q	M	36	46	10	Hom c.1529C>T	Hom p.Ala510Val	Missense
24	R	M	46	59	13	c.1529C>T c.1672A>T	p.Ala510Val p.Lys558	Missense Nonsense
25	S	M	45	78	33	c.1529C>T c.1617delC	p.Ala510Val p.Val540fs	Missense Frame shift
26	T	M	33	42	9	Hom c.1529C>T	Hom p.Ala510Val	Missense
27	U	F	42	52	10	c.1529C>T c.1939del	p.Ala510Val p.Ala647fs	Missense Frame shift
28	V	M	34	72	38	Hom c.1529C>T	Hom p.Ala510Val	Missense
29	W	F	43	52	9	c.1529C>T c.2228T>C	p.Ala510Val p.Ile743Thr	Missense Missense
30	Y	M	53	61	8	Hom c.1529C>T	Hom p.Ala510Val	Missense
31	Z	M	20	44	24	c.861dup c.1529C>T	p.Asn288* p.Ala510Val	Frame shift Missense
32	Z	M	26	43	17	c.861dup c.1529C>T	p.Asn288* p.Ala510Val	Frame shift Missense

#Symptomatic individual with suspected *SPG7*-associated disease

Taken from [212]

Table 6.2 Clinical characteristics of individuals with *SPG7*-associated spastic ataxia

Pt															
	Ocular findings	Visual acuity		Ishihara test plate errors*	Dysarthria	Gait ataxia	Limb ataxia	Spasticity LL	Reflexes LL	Plantar responses	BBD	NP	Other	SARA	Cerebellar atrophy
1	Saccadic pursuits	20/20	20/20	0	+	+	+	++	Increased	↑↑	Bladder	–	–	10.5	+
2	GEN, slow saccades	20/20	20/30	1	+	+++	+	+	Increased	↑↑	Bladder & Bowel	–	–	19	Y
3	↓ upgaze, GEN, hypermetric saccades	20/25	20/25	0	++	++	+	+++	Increased	↑↑	Bladder	–	–	9	++
4	GEN	20/20	20/25	0	++	+	+	+++	Increased	↑↑	Bladder & Bowel	–	–	13.5	++
5	mild disc pallor	20/20	20/20	0	++	+	+	+++	Increased	↓↓	–	–	–	7	+
6	GEN, hypermetric saccades	20/20	20/20	0	+	+	+	+	Increased	↑↑	Bladder	–	–	10.5	++
7	N	20/20	20/20	1	–	+	+	++	Increased	↑↑	Bladder	–	–	3	+
8	↓ upgaze, GEN, slow saccades	20/30	20/30	0	++	++	++	+	Increased	↑↓	–	–	–	12	+++
9	Visual loss	LP	LP	N/P	+	+	+	–	Increased	↓↓	–	N/P	JMD	*	+
10	PEO, ptosis, cataracts, disc pallor	20/40	20/50	N/P	+++	+++	++	+++	Increased	↓↓	Bladder & Bowel	–	Dysphagia, ↑lactate, ↑pyruvate	28.5	++
11	N	20/20	20/20	2	+	+	+	++	Increased	↓↓	Bladder	–	–	6.5	++

12*	N	20/40	20/40	12	++	+++	+	—	N	↓↓	Bladder	—	—	10	++
13	GEN, sacc pursuits, hypometric saccades	20/20	20/20	1	—	++	+	+	Increased	↑↓	—	—	—	7	+
14	GEN, sacc pursuits	20/20	20/20	1	—	+	+	+++	Increased	↑↑	Bladder & Bowel	+	Waddling gait	8	+
15	N	20/20	20/20	2	—	+	—	++	Increased	↑↑	Bladder & Bowel	—	Waddling gait	3.5	+
16	Slow saccades, disc pallor	20/25	20/25	15	++	+	+	++	Increased	↑↓	—	—	COG, Retinal pigment, telangiectasia, Dysphonia	13	++
17	↓ upgaze, GEN, sacc pursuits	20/20	20/25	0	+	++	+	+	Increased	↑↑	Bladder	—	—	11	+
18	GEN, hypometric saccades	20/25	20/25	0	+	++	+	+	N	↓↓	Bladder & Bowel	—	—	11	+
19	↓ lat gaze	20/20	20/20	1	+	+	+	++	Increased	↑↑	Bladder	+	COG, Deafness	11.5	N/P
20	N	20/30	20/25	0	—	+	—	++	Increased	↑↑	Bladder & Bowel	—	—	5	+
21	GEN, sacc pursuits	20/20	20/20	0	—	+	—	+	Increased	↑↑	Bladder & Bowel	—	—	4	N/P
22	↓ upgaze, GEN, sacc pursuits, slow saccades	20/25	20/20	0	—	++	+	+++	Increased	↑↑	Bladder	—	—	9.5	+
23	GEN	20/20	20/25	3	++	+	+	++	Increased	↑↑	Bladder	N/P	—	11.5	++

24	↓ lat gaze, slow saccades	20/80	20/25	1	+	++	+	++	Increased	↓↓	Bladder	N/P	–	12	++
25	PEO, ptosis	20/25	20/40	20	+++	+++	+	+++	Increased	↑↑	Bladder	N/P	–	29	Y
26	Sacc pursuits, hypometric saccades	20/30	20/20	2	–	+	+	++	Increased	↑↓	–	–	–	5.5	+
27	GEN	20/25	20/20	0	+	++	+	+	Increased	↑↑	Bladder	–	–	8	++
28	↓ lat + up gaze, slow saccades	20/25	20/20	1	++	+++	+++	+	Increased	↓↓	Bladder	–	COG	23.5	++
29	GEN, sacc pursuits, Slow saccades, ↓ up and hor gaze	20/20	20/25	2	–	+	–	++	Increased	↑↑	Bladder	–	–	8.5	++
30	N	20/20	20/200	0	–	+	+	++	Increased	↑↑	Bladder & Bowel	–	–	4.5	+
31	Sacc pursuits, hypometric saccades	20/20	20/20	0	+	+	+	+	Increased	↑↑	Bladder	–	Dysphonia	4.5	++
32	GEN, sacc pursuits, Slow saccades	20/25	20/20	N/P	++	+	+	+	Increased	↑↑	–	N/P	–	7.5	N/P

GEN, Gaze- evoked nystagmus; PEO, Progressive external ophthalmoplegia; Sacc pursuits, Saccadic intrusions on pursuit; Telangiectasia, conjunctival telangiectasia; JMD, Juvenile macular degeneration; LP, Light perception; COG, Cognitive impairment; BBD, Bladder and bowel dysfunction; LL, Lower limbs; SARA, Scale for the assessment and rating of ataxia (0–40); Visual acuity (Snellen), presented as best- corrected VA; NP, Neuropathy; N/P, not performed; + Mild, ++ Moderate, +++ Marked; * Normal colour vision ≥ 17 plates read normally; # Symptomatic individual with suspected *SPG7*-associated disease; Y, MRI cerebellar atrophy reported by radiologists, images not available for review by neurologists. Taken from [212]

All patients had variable degree of ataxia and all but two had spasticity (93.7%). Two individuals had spasmodic dysphonia, while one patient was blind and could not complete the SARA. For the remaining 31 patients mean SARA score was $9/40 \pm 6.4$ (range 3-29).

Among the 27 individuals who had neurophysiology (mean age 57.1 ± 10.3 , mean disease duration 17.4 ± 9.7 years), 2/27 (7.4%) had evidence of a neuropathy; length-dependent large-fibre axonal sensory in one and a sensory-motor neuropathy in the other (nerve conduction studies shown in Table 6.3). NCS in the remaining 25/27 were normal, including those with symptom onset over two decades ago.

Table 6.3 NCS data on two patients with neuropathy

Patient (age at assessment)		14 (47)			19 (70)	
Motor Nerves	Lat (ms)	Amp (mV)	CV (m/s)	Lat (ms)	Amp (mV)	CV (m/s)
Right Median	4.56	5	50	3.97	10	50.7
Right Peroneal	7.42	2.4	37			
Right Tibial	4.83	5.8	36	4.34	7.9	40.3
Sensory Nerves	Lat (ms)	Amp (μV)	CV (m/s)	Lat (ms)	Amp (μV)	CV (m/s)
Left Median	Digit III - wrist	3.35	5	35		
Right Superficial Radial	Forearm - snuffbox	1.38	15	65	1.74	11
Left Ulnar	Digit V - wrist	2.44	4	57		
Left Sural		NR		3.94	4	29.4

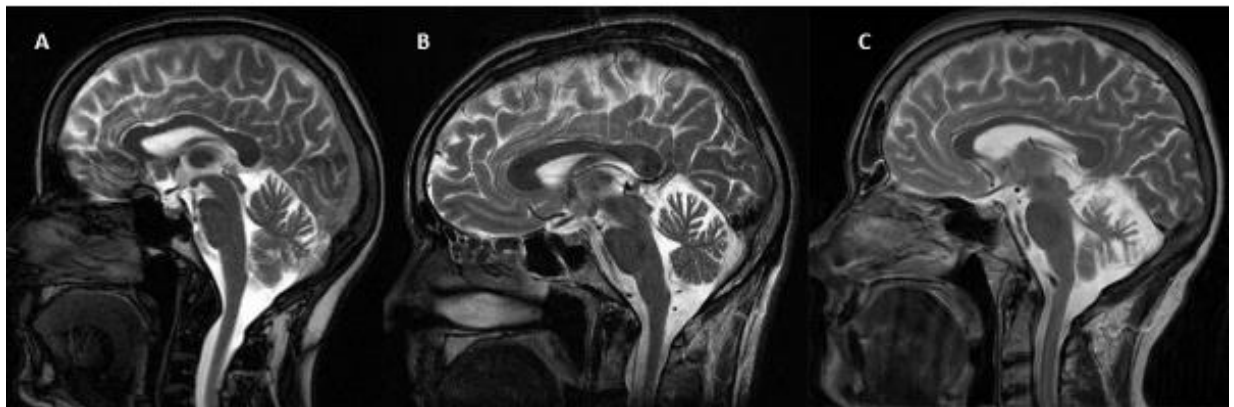
Taken from [212]

NR, Not recordable; Abnormal values highlighted in **bold**; Lat, Latency; Amp, Amplitude; CV, Conduction velocity; ms, Milliseconds; mV, Millivolts; m/s, Metres per second; µV, Microvolts

Normative values are shown in Appendix 8

MRI brain (n=29) demonstrated variable degree of cerebellar atrophy. In the majority of cases both anterior and posterior lobes were involved. Similarly both vermis and cerebellar hemispheres were involved in all patients (Table 6.2, Figure 6.1).

Figure 6.1 Magnetic resonance images of three patients with *SPG7*-associated disease



Sagittal T2 views thorough the corpus callosum. Both anterior and posterior lobe, and both vermis and cerebellar hemispheres atrophy is observed. Cerebellar atrophy was graded by consensus as: **A**, mild (patient 20); **B** moderate (patient 27); **C**, marked (patient 8)

6.4.2 Ophthalmological findings

VA was reduced in *SPG7* patients compared to controls, but did not reach statistical significance ($p=0.08$). In 26 of 32 patients VA was 20/30 or better with an average Snellen VA 20/25 (range 20/20 to light perception).

Colour vision assessment demonstrated red-green colour vision deficiency in 2/29, and total colour blindness in 1 individual. Remaining 26 patients had normal testing, although 12 subjects made between 1-3 errors. Fundus examination revealed disc pallor in 3/32 and retinal pigmentation in 2/32. The majority of *SPG7* patients ($n=25$, 78.1%) had abnormal ocular motor findings. Detailed characteristics of individuals with *SPG7*-associated spastic ataxia are presented in Table 6.2.

6.4.3 Optical coherence tomography

The mean age at baseline OCT assessment was 56.8 ± 10.7 (range 39 - 78 years). In total 8 eyes were excluded from RNFL analysis due to movement artefacts or poor image quality.

The results of OCT studies are summarised in Table 6.4. The average peripapillary RNFL and foveal thickness in *SPG7* were not different from controls. RNFL thickness in the temporal quadrant was reduced in patients, compared to controls ($p<0.05$), while nasal, inferior and superior quadrants did not demonstrate abnormal RNFL thinning. 20% of individuals had temporal RNFL thickness below the 95% lower limit of normal, defined in the normative database provided by the manufacturer. In addition, significant reduction of foveal thickness (patient 9) and macular degeneration (patient 25) were identified. There was no statistically significant difference in OCT findings between

individuals with at least one null variant (n=12) and those with missense variants (n=20), p=0.5.

OCT measurements of asymptomatic heterozygous *SPG7* first-degree relatives were not statistically different from affected individuals' data and controls (Table 6.4).

Of the three individuals with ataxia of undermined cause and heterozygous *SPG7* mutation, one had disc pallor on fundoscopy and significant temporal RNFL thinning (35µm) with sectorial RNFL thickness below the normal limits in all except the nasal quadrant. This group had significantly reduced average RNFL thickness in comparison to controls (p=0.01), while OCT findings in the asymptomatic individual with two *SPG7* variants were within normal limits.

Table 6.4 RNFL thickness results in patients with *SPG7*-associated spastic ataxia

	Controls	<i>SPG7</i>	Asymptomatic Het <i>SPG7</i>	Symptomatic Het <i>SPG7</i>	Asymptomatic comp het <i>SPG7</i>
N (females)	32 (13)	32 (9)	4 (3)	3 (1)	1 (1)
BCVA (LogMAR)	0.05 ± 0.07	0.16 ± 0.49 p=0.08	0.03 ± 0.04 p=0.4	0.45 ± 0.77 p=0.3	0
Age (years) Mean ± SD (range)	55.4 ± 9.1 (39 - 73)	56.8 ± 10.7 (39 - 78) p=0.6	58 ± 21.7 (27 - 74) p=0.4	62.3 ± 7.1 (56 - 70) p=0.3	66
OCT Foveal (µm) Mean ± SD (range)	243.3 ± 32.6 (201.5 - 308.5)	241.7 ± 23.8 (201 - 303) p = 0.8	229.8 ± 19.2 (212 - 254) p=0.3	244.3 ± 23.5 (219 - 267) p=0.4	229
OCT Average (µm) Mean ± SD (range)	102.6 ± 8.6 (83 - 124)	101.5 ± 7.1 (82 - 114) p=0.6	95.1 ± 21.4 (70 - 121) p=0.5	82.7 ± 19.6 (56 - 95) p=0.01^{##}	104
OCT Superior (µm) Mean ± SD (range)	118.4 ± 13.2 (89 - 140)	118.1 ± 12.3 (82 - 141) p=0.9	115.5 ± 19.6 (68 - 163) p=0.9	100.3 ± 23.9 (64 - 117) p=0.3	123.5
OCT Nasal (µm) Mean ± SD (range)	90.4 ± 10.7 (71 - 114)	91.5 ± 11 (68 - 110) p=0.8	79.3 ± 26 (49 - 108) p=0.4	68.7 ± 12.5 (52 - 82) p=0.6	93.5
OCT Inferior (µm) Mean ± SD (range)	126.6 ± 13.5 (109 - 146)	123.1 ± 10.7 (101 - 145) p=0.3	120 ± 34.7 (100 - 146) p=0.6	104.8 ± 25.8 (70 - 124) p=0.3	126
OCT Temporal (µm) Mean ± SD (range)	75.2 ± 9 (60 - 93)	70.3 ± 10 (40 - 81) p=0.04[#]	65.9 ± 18 (59 - 79) p=0.2	57.2 ± 19.6 (32 - 72) p=0.3	72.5

#ANOVA with Tukey; ##Kruskal-Wallis with Dunn's

Taken from [212]

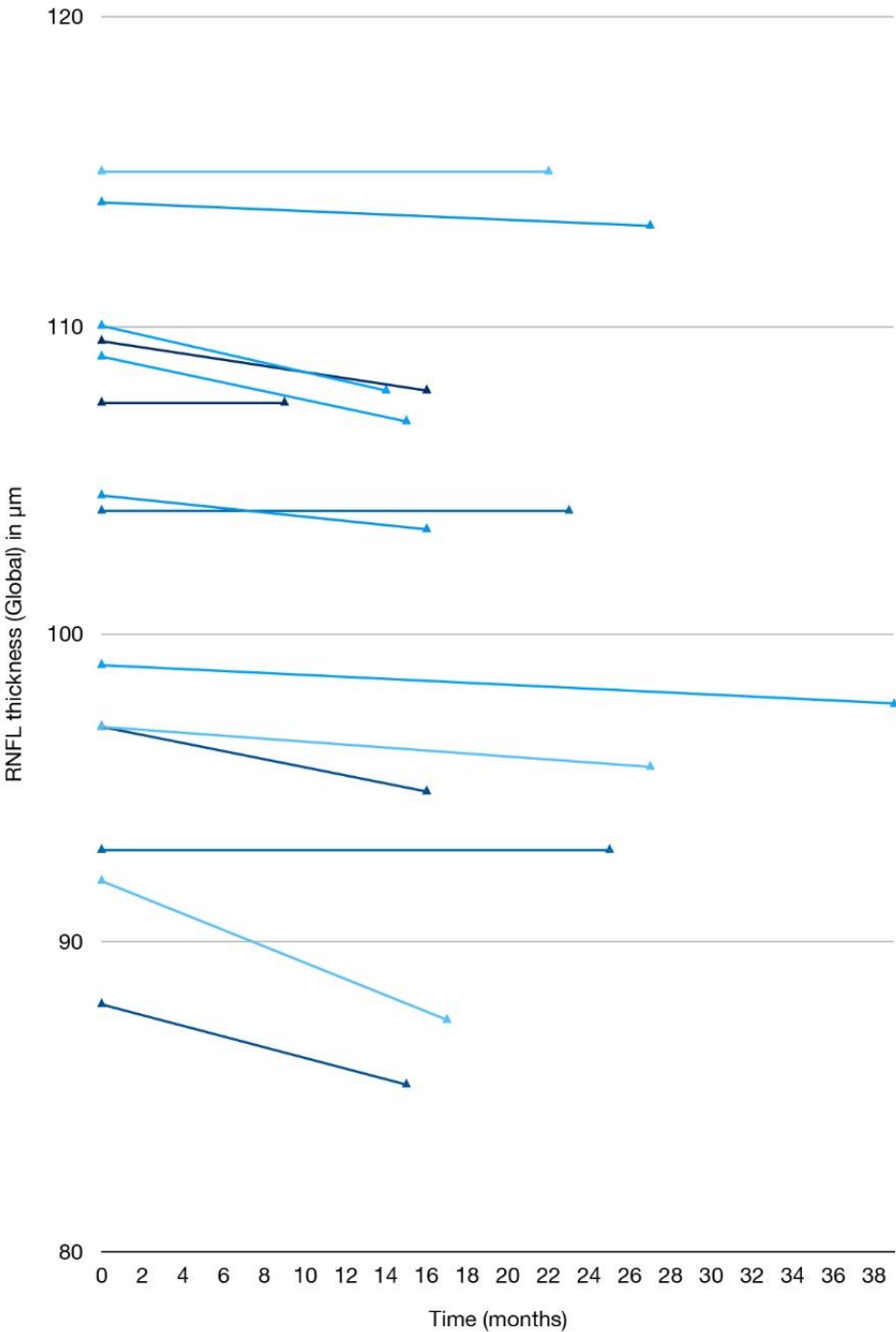
6.4.4 Correlation with clinical features

There was no significant correlation between baseline foveal, average or RNFL thickness in different sectors and disease severity as quantified by SARA ($r_{\text{Average RNFL vs SARA}} = -0.075$, $p=0.7$; $r_{\text{Temporal RNFL vs SARA}} = -0.17$, $p=0.4$) or disease duration ($r_{\text{Average RNFL vs duration}} = -0.031$, $p=0.8$; $r_{\text{Temporal RNFL vs duration}} = 0.022$, $p=0.9$). Moreover, there was no significant correlation between RNFL thickness and visual function in these two groups ($p=0.7$).

6.4.5 Longitudinal assessment

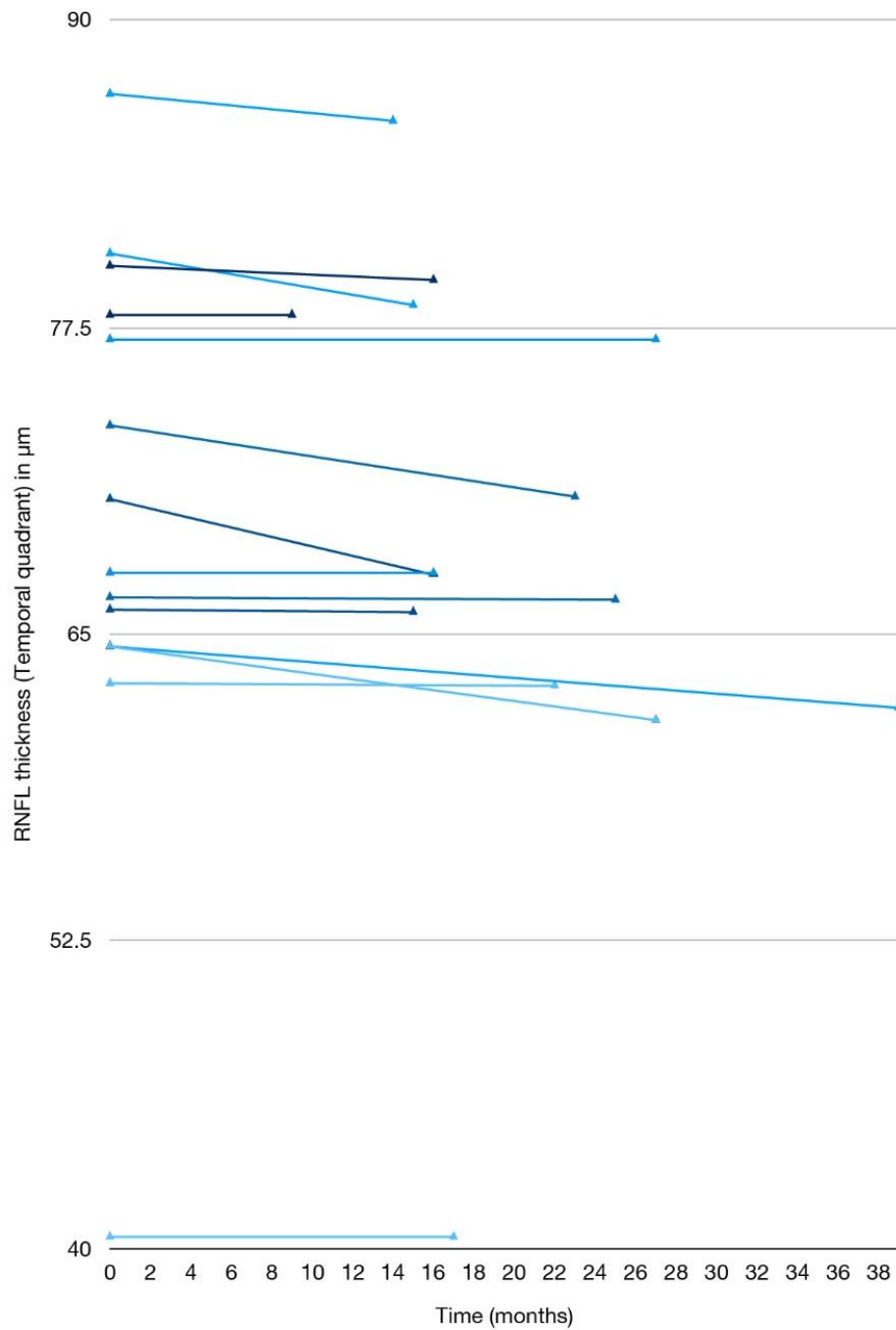
14 individuals had further OCT studies during a mean follow-up interval of 20.1 months (range 9-39). There was significant decline in average (Figure 6.2) and temporal RNFL (Figure 6.3) thickness over time ($n=10$, $p<0.05$), both in individuals with missense and null variants. Disability progression, documented in 11/14 patients, is shown in Figure 6.4 (mean SARA at baseline 9.4 ± 4.2 , at follow-up 11.5 ± 4.7 , $p<0.05$). Most individuals had advancement in gait and fast alternating hand movements (6 patients each) and worsening in the heel-shin slide (5/11), while stance and speech changes were seen in 4 patients each.

Figure 6.2 Global RNFL thickness at baseline and at follow-up evaluation



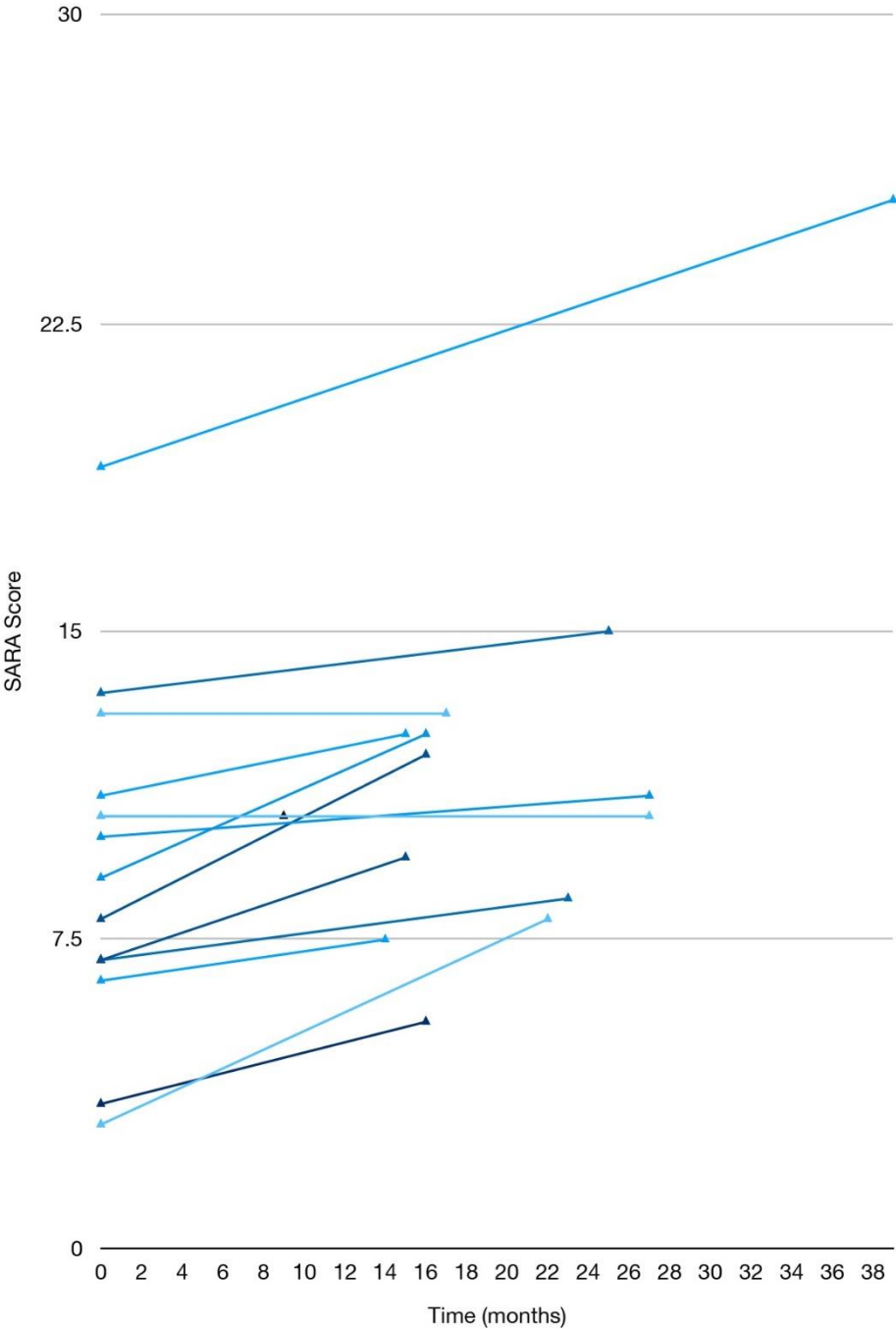
Taken from [212]

Figure 6.3 Temporal RNFL thickness at baseline and at follow-up evaluation



Taken from [212]

Figure 6.4 SARA score at baseline and at follow-up assessment



Taken from [212]

6.4.6 Genetic analysis

Fifteen different *SPG7* variants were identified (Table 6.1). The missense c.1529C>T (p.Ala510Val) was the most frequent variant (21 families, n=26, 84%), present in homozygous state in 57% of the families (12/25, n=16). Compound heterozygous *SPG7* mutations were found in 12/25 (n=15) and in 8/12 p.Ala510Val was in association with a second *SPG7* variant.

Five *SPG7* variants are novel and four are considered pathogenic according to American College of Medical Genetics (ACMG) guidelines: the frameshift p.Pro350Glnfs*36, p.Ala647fs and p.Val540fs (previously reported by the Mater Misericordiae University Hospital group) [231], and the missense p.Pro749Leu. The p.Val594Ala variant, classified as VUS (Variant of uncertain significance), is localised at a highly conserved position within a functionally important domain of the protein. Although we are unable to provide proof of pathogenicity, this variant is in compound heterozygosity with another *SPG7* pathogenic variant and associated with near-identical clinical findings to the other *SPG7* patients and therefore is suspected to be relevant.

Null mutations (on one allele, 12/32 patients) were associated with earlier symptom onset (32.6 vs 43 years, p=0.02) and patients assessed presented with longer disease duration (22.5 vs 14.9, p=0.03) compared with missense variants. There was no significant difference in symptom onset age between individuals with homozygous p.Ala510Val and compound heterozygous variants where p.Ala510Val was in association with another missense variant (n=3).

Two individuals who carried null mutations (patients 4 and 6) had symptom onset in their teens and very mild disease course over 3 to 4 decades, while patients 10 and 25 with symptom onset in middle age and more complex phenotype had significant disease

progression. Dysarthria was observed in all, but one family with null mutations (9/12, 75%), and was variably present in patients with missense variants (13/20, 65%), $p=0.56$. Dysphonia was present in one of otherwise very similarly affected siblings (family Z) who carried a null mutation, as well as in one individual with missense variant, while cognitive decline was documented in three individuals with homozygous p.Ala510Val, including a single sibling from the large family O.

All 3 individuals in whom SARA score remained unchanged over time carried at least one p.Ala510Val, in compound heterozygosity with the frameshift p.Phe284fs in one. Interestingly, most significant disease progression (SARA from 19/40 to 25.5/40) was observed in the single individual with advancement in the sitting item, who carries the compound heterozygous variants p.Gly349Ser and p.Gly352fs.

Of the three heterozygous *SPG7* individuals with progressive ataxia or HSP, two had p.Ala510Val (no copy number variations on MLPA), and one had p.Phe284Profs*45 shared with an unaffected sibling.

6.5 Discussion

This is the first comprehensive phenotyping exercise involving Irish patients with *SPG7*-associated spastic ataxia, demonstrating significant phenotypic variability and novel clinical features. To our knowledge this is the largest cohort description of the peripapillary RNFL measures as detected by OCT [130, 131, 232] and the biggest neurophysiological study in paraplegin deficiency [131, 160, 161, 233]. Worldwide, *SPG7* has been reported as one of the most frequent causes of ARCA, being the fourth commonest cause of genetic ataxia in the UK [25] and it is the second most common ataxia in the NAC where the largest cohort of patients with ataxia in Ireland is followed [5].

Although *SPG7* mutations were originally linked to HSP [229], all individuals in this cohort displayed ataxia, which in contrast to prior reports, was more pronounced than spasticity in only a quarter of them [151]. This may of course reflect a referral bias to the National Ataxia Clinic from where the majority of patients were recruited. The clinical spectrum associated with *SPG7* was highly variable even within pedigrees. It is unclear how a single mutation can cause the wide diversity of clinical features in individuals sharing the same variant, but significant phenotypic heterogeneity is seen in other conditions, especially mitochondrial, even among individuals of the same pedigree [214]. It is possible that additional genetic, epigenetic, and environmental factors modify the phenotypic expression of p.Ala510Val, the most frequent variant in this cohort. Although the overall phenotype was of slowly progressive complex spastic ataxia, additional features were generally mild. None of our patients had parkinsonism, recently reported in about one fifth of *SPG7* cases even as initial presentation [234].

Peripheral neuropathy, frequently documented in other spastic ataxias, including FRDA and ARSACS, as well as in most common SCAs [235], was reported in some *SPG7* cohorts [131, 160, 233], but not observed in others [161]. In comparison, axonal neuropathy was identified in only 7% of these patients, associated both with null and missense variants and was not genotype-specific since other affected siblings had normal NCS. Animal studies demonstrated that paraplegin deficiency causes late onset distal axonopathy involving long axons of the PNS and CNS [236] and clinically, optic neuropathy has been detected in individuals with more advanced *SPG7*-related disease [131]. Interestingly, in our cohort peripheral neuropathy was not identified even in patients with disease duration of over 30 years, indicating that recessive *SPG7* mutations may account for spastic ataxia with peripheral neuropathy in only a small proportion of patients.

Notably, although paraplegin is ubiquitously expressed, its deficiency affects only a specific subset of mitochondria and leads to selective degeneration of a subset of axons [236], which might explain the phenotypic variability in this *SPG7* cohort. All but one of the patients had predominant involvement of the corticospinal tracts. A study of paraplegin-deficient mice indicated that *SPG7* causes late onset axonopathy of spinal, optic and peripheral axons with mild muscular involvement and demonstrated that axonal degeneration begins distally and slowly moves proximally along the axon. Structurally abnormal mitochondria in distal regions of affected axons was documented at 4.5 months of age and at 8 months, approximately 20% of axons of the same tracts were filled with abnormal mitochondria. Occasional axonal swellings were seen initially at 8 months with increasing numbers by 1 year of age in association with slow, progressive loss of fibres due to degeneration. Axonal degeneration became prominent at 15 months, indicating that the neurologic impairment of paraplegin-deficient mice

observed in the early stages is not directly due to the loss of axons. Paraplegin-deficient mice initially develop normally with the first signs of motor impairment demonstrated by difficulty maintaining balance on the rotarod seen at 4 months of age, progressive decline in rotarod performance follows. The phenotype is characterised by an abnormal gait with uncoordinated movement of the hindlimbs by the age of 17 months, when mice also have a pronounced scoliosis, while a statistically significant loss of body weight is documented at 12 months [236].

Although well described, optic nerve atrophy, recognised as a disc pallor on fundus examination, has been documented in a relatively small proportion of *SPG7* cases [151, 160, 161, 229, 230], and is seen in only 6% of this cohort. In a French study, of 10/23 who had evidence of optic neuropathy on OCT, 40% had normal-appearing optic discs on fundoscopy [131]. In another small study only individuals with complex *SPG7*-associated phenotype had RNFL loss detected by OCT [130], alike this cohort comprising complex cases. Direct comparison of measurements between various studies is difficult due to the differences in OCT devices used. However, 13 previously reported AR *SPG7* cases with optic neuropathy detected by OCT, documented diffuse and sectorial, particularly temporal RNFL loss [130, 131, 232], similarly to our findings of predominantly temporal and progressive RNFL reduction.

This study demonstrated the benefit of OCT, a non-invasive, easily applicable and reproducible technique for evaluation in real time [45] to detect RNFL thinning even in individuals with no visual impairment. OCT has previously indicated potential to differentiate diseases with similar characteristics [141] implying that specific RNFL patterns might be of diagnostic value in heterogeneous and often overlapping phenotypes. Although abnormal RNFL thickness has been documented in other spastic ataxias, contrary to *SPG7* a distinct thickening of the RNFL is seen in ARSACS [91].

OCT findings in FRDA, another entity in which mitochondrial dysfunction has a central role, show predominantly average and superior quadrant RNFL loss, suggesting that optic neuropathy in FRDA is likely to involve different disease mechanisms leading to diverse areas of selective vulnerability [34, 101]. The pattern of predominantly temporal RNFL reduction has also been reported in SCA1 [105], typically early-adulthood onset ADCA, but was not observed in another SCA1 study [38].

Individuals with *SPG7* may develop a range of visual features, including red-green colour vision deficiency. Decreased RNFL thickness predominantly in the temporal sector, which consists primarily of the highly specialised in colour discrimination P-cells, may explain dyschromatopsia in the *SPG7* patients. Similarly, impaired colour vision has been reported in Parkinson's disease, where significantly reduced temporal RNFL thickness has also been documented [237]. In contrast, individuals with MSA-C, considered the most common idiopathic late-onset CA, have predominantly decreased global and inferior RNFL thicknesses [238] and colour vision is usually unaffected. These data indicate that colour vision testing and OCT can be useful additional tools to distinguish between *SPG7* and MSA-C, two typically late-onset presentations.

All affected individuals who displayed colour blindness had at least one p.Ala510Val variant, but it is unclear if this can explain the dyschromatopsia observed only in a small proportion of patients. It is uncertain when the red-green colour blindness became evident and it would be interesting to see if a correlation between colour vision and disease duration can be established over time in those who made errors on Ishihara testing.

Optic atrophy with dyschromatopsia has been described in other mitochondrial disorders with primary involvement of P-cells, including Leber's hereditary optic neuropathy

(LHON) and dominant optic atrophy (DOA). In both entities early and preferential involvement of the papullo-macular bundle [40, 211] leads to characteristic temporal loss, similarly to our findings, but without associated non-ophthalmologic features. Furthermore, optic neuropathy is frequently reported in other multi-systemic mitochondrial disorders [40, 214], including CMT2A, the most common type of AD axonal CMT due to mutations in *Mitofusin 2*, a protein on the outer mitochondrial membrane [239]. In contrast, m-AAA mitochondrial protease paraplegin co-assembles with the homologous protein, AFG3L2, to form a complex in the mitochondrial inner membrane [240]. This complex plays an important role in various mitochondrial processes and mutant paraplegin fails to process proteins phosphorylated by AFG3L2 leading to toxic accumulation of reactive oxygen species, a general paradigm of the mitochondrial optic neuropathies [211]. As a result of the paraplegin assembly with highly homologous AFG3L2, abnormal levels of the SPG7 protein can impact the function of the m-AAA protease complex in the cerebellum and therefore missense and null mutations can have a different phenotypic effect [160]. Consequences of null *SPG7* variants leading to absent or severely truncated protein can be explained by the ability of AFG3L2 to still form functional homo-oligomeric m-AAA protease, while missense variants such as p.Ala510Val, resulting in a defective protein product, may form dysfunctional heteromeric complexes with AFG3L2 [233].

In addition to reported recessive *SPG7* phenotypes, DOA has also been described, indicating that some heterozygous *SPG7* mutations can cause isolated optic neuropathy with documented RNFL loss mainly in the temporal quadrant [131, 241]. Similarly to recessive *SPG7* cases, relatively mild visual deficits have been reported in individuals with DOA due to single *SPG7* mutations contrasting with the more severe visual loss

observed in patients with non-syndromic DOA associated with heterozygous *AFG3L2* variants, traditionally linked to AD SCA28 [131, 241].

One individual displayed blindness due to early onset macular degeneration, also shared by a sibling unaffected with ataxia, but not present in a sibling with spastic ataxia (family H), which suggests a possible second inherited condition. Animal studies at different ages showed optic nerve involvement with axonal swelling in paraplegic-deficient mice, but no degenerating axons in younger animals, indicating that optic nerve presentation arises later [236]. However, neuropathology of a 70-year old *SPG7* patient with severe visual loss since childhood, long before gait disturbance, showed significant optic system degeneration with severe atrophy of the optic nerves, chiasm and optic tracts with an almost complete loss of axons and myelin sheaths in combination with severe astrogliosis [160].

Interestingly, retinal findings in the subgroup of individuals with ataxia of undermined cause and a heterozygous *SPG7* variant were different compared to individuals with *SPG7*. However, as the number of patients in this subgroup is very small, the significance of this finding is unclear. Our results suggest that the identified heterozygous *SPG7* variants are likely irrelevant to their presentation and that optic neuropathy is caused by an alternative underlying pathology, which is yet to be determined.

Over 75 different *SPG7* mutations have been identified to date [161, 230]; Ala510Val is considered the commonest *SPG7* mutation worldwide with a relatively high carrier frequency in the general population (1.5%-3%) [152, 160, 242]. Among the families in this cohort the frequency of the Ala510Val was 62% (31 of 50 alleles assessed), which is in accordance with other studies (58.5%-60%) [161, 233]. In contrast to the largest *SPG7* cohort reported to date [233] suggesting later onset in patients carrying at least

one Ala510Val variant, in this study the two patients with earliest symptom onset (12 and 15 years, respectively) both carried this variant in a heterozygous state.

We did not find correlation between RNFL and disease severity as measured by SARA, similarly to HSP studies, where no correlation was found between RNFL thickness and disease severity assessed by SPRS [130, 132]. While SARA score evaluates the ataxic manifestations, the SPRS quantifies the functional impairment of spastic paraplegia, suggesting that both scales have limitations in objectively estimating progression in the broader phenotypic spectrum of *SPG7*. These limitations might be addressed by OCT and my findings support the potential of this technique to become a useful tool for measuring disease progression in *SPG7*-associated spastic ataxia in addition to detecting subclinical optic neuropathy, which appears to be common in *SPG7*.

6.6 Conclusion

This study provides neurophysiological and retinal data to help refine the phenotype of this increasingly recognised spastic ataxia. Furthermore, this study highlights that *SPG7* mutations may account for spastic ataxia with peripheral neuropathy in only a small proportion of patients. RNFL abnormalities in the temporal quadrant, despite clinical heterogeneity, are common even in the absence of decreased visual acuity and OCT should be considered part of the routine evaluation in HSP and CA with further work being required to clarify its value as a potential biomarker of disease progression in *SPG7*-related disease.

Chapter 7: Clinical phenotype and optical coherence tomography in autosomal dominant cerebellar ataxias and complex autosomal dominant disorders associated with ataxia

7.1 Introduction

7.1.1 Spinocerebellar ataxias (SCAs)

The SCAs are a diverse group of slowly progressive neurodegenerative ataxic disorders with autosomal dominant (AD) pattern of inheritance, presenting with ataxia of gait, stance and limbs, dysarthria and / or oculomotor disorder due to cerebellar degeneration. To date, more than 40 genetically distinct subtypes have been described. SCAs are designated by a number indicating the chronological order in which the disease locus was first identified and the most recently described subtype is SCA48 [102]. This classification is imperfect, as SCA9 has never been related to any clinical disorder; whereas SCA15 and SCA16 share the same gene, as well as SCA19 and SCA22 [243]. Pathogenetic classification divides SCAs into two main groups: polyglutamine (polyQ) SCAs, caused by CAG nucleotide repeat expansions that encode polyglutamine and, therefore, involve the toxic polyglutamine protein (polyQ), and SCAs, caused by standard mutations (non-repeat expansion). There are seven polyglutamine SCAs in which protein misfolding leads to intraneuronal inclusions and accelerated cell death in many parts of the central nervous system. These are the most frequent SCAs worldwide [21, 22] with SCA1, 2, 3, 6 and 7 representing approximately 80% of ADCA. The age of symptom onset is inversely related to CAG repeat length [244].

Differentiating the various types of SCA based on clinical manifestations alone is difficult. While no individual sign is pathognomonic, previous reports demonstrated that some ophthalmic features are more prevalent in specific SCAs and, in combination with other clinical manifestations in affected individuals, can aid distinction of different SCA subtypes. The efferent system is most commonly affected, but subclinical or clinical involvement of the afferent visual system has also been observed [103] with optic nerve dysfunction reported in patients with various subtypes of SCA, e.g. SCA1, SCA2, SCA3, and SCA7 [38, 106, 111].

The use of OCT has been investigated in some SCA subtypes. Retinal changes as detected by OCT, including measurement of the thickness of the retinal nerve fibre layer (RNFL) and other layers of the retina, have been described in most common trinucleotide expansion SCAs (SCA1, SCA3, SCA7) and in mixed SCA cohorts.

A study of nine patients with SCA1, a subtype of SCA which causes significantly faster functional decline than other SCAs [104], reported statistically significant average RNFL loss compared to healthy controls with most prominent reduction in the temporal region [105]. However, these findings were not replicated in another study which included seven individuals with SCA1 [38]. In contrast, peripapillary RNFL thickness was also reduced in patients when compared to controls in a larger study of 20 SCA1 individuals [106].

Macular dysfunction in association with SCA1 has also been documented, initially in a small number of patients [107, 108]. A further study of 20 individuals with SCA1 reported that 25% of patients displayed a distinct maculopathy, characterised by a disruption of the ellipsoid zone (EZ disruption), suggesting retinal involvement in this entity in addition to the widespread neurodegeneration [106].

In one of the studies, Stricker et al. [105] did not find a correlation between RNFL thickness and disease duration, disease severity measured with SARA score and visual acuity in SCA1 patients.

In the literature, there is limited data on retinal findings as detected by OCT in patients with SCA2 and SCA3. Mild reduction in RNFL thickness as detected by OCT has been reported in 9 patients with SCA3. In 15 of 18 eyes assessed the average RNFL thickness was lower than the population average [109]. Temporal sector thickness was preserved in all, but reduction in the superior, inferior and nasal sectors has been identified in some eyes.

In the same study, a negative correlation was found between RNFL thickness and SARA score, but there was no significant relationship between RNFL measures and disease duration.

In a mixed SCA study of patients with SCA1 (7 patients), SCA2 (7 patients), SCA3 (5 patients) and SCA6 (5 patients) [38] subjected to OCT, average RNFL thinning was observed in SCA2 and SCA3 groups only, when compared to control subjects. Furthermore, this study reported that the overall thickness in the macular region was significantly thinner in SCA1, SCA3 and SCA6, but not in SCA2 subgroups.

Disease severity as quantified with SARA score was inversely correlated with the OCT measurements in the SCA2 and SCA3 subgroups.

In a comparative OCT study of individuals with SCA3 (10 patients), and SCA10 (9 patients) [110], RNFL had a tendency to be thicker in patients with SCA10 than in those with SCA3, but did not reach statistical significance. Changes in RNFL were observed in at least one region in 6/10 individuals with SCA3 and in only 2/9 SCA10 patients. Retinal changes in affected SCA3 cases demonstrated significant nasal RNFL thinning,

which was inversely proportional to SARA score used to quantify disease severity. Overall, the findings from this study suggested that in SCA10 RNFL thickness changes are less prominent and unrelated to disease duration.

Disease-specific involvement of the afferent visual system, although seen in various SCAs, is most prominent and well described in SCA7 [111]. Visual loss, blue–yellow colour vision defects and blurred vision may occur years before other manifestations of the disease. The central vision becomes affected first and can progress to complete blindness. Retinal changes might be very subtle initially, affecting the central macula, and subsequently advance to cone-rod dystrophy, the underlying cause of progressive vision loss in SCA7 [112].

Retinal thinning with loss of the peripapillary RNFL has been demonstrated by OCT in SCA7 patients with gradual loss of vision [113]. A family OCT study has revealed that the retinal thinning extends outside the visibly atrophic lesions [114].

A recent OCT study included 16 patients with SCA7 who underwent a comprehensive ophthalmic examination, including OCT of the optic nerve and macula [115]. Unsurprisingly, all 9 individuals who had topographic macular analysis had various degree of maculopathy with foveal atrophy present in all, and normal average RNFL thickness in majority of patients. No correlation was sought between retinal changes and disease severity, but an inverse association was found between SARA score and endothelial corneal cell density.

These results were replicated in another study where foveal thinning on OCT was documented in all 13 symptomatic SCA7 cases [116], but not in controls (n = 5) or pre-symptomatic carriers (n = 3). Interestingly, in this study visual fields and Spinocerebellar Ataxia Functional Index (SCAFI, Appendix 2) [117] were significantly correlated with

time-to-disease onset (pre-symptomatic) / disease duration (symptomatic carriers) and SCAFI showed a trend to differentiate pre-symptomatic carriers from controls.

Structural changes in the retina and optic disc as detected with an OCT have been rarely reported in other types of SCAs.

SCA14, caused by a conventional mutation in the *PRKCG* gene, has an estimated incidence rate 1-4% in ataxia populations with more common SCAs excluded [118]. The protein kinase C gamma is expressed in neurons of the brain and the spinal cord, particularly in Purkinje cells. It is also found in the visual system and especially in the retina [119]. A study investigated the visual function as well as structural change in the afferent visual system in 12 SCA14 individuals compared to age-matched controls [120]. Although visual acuity and contrast sensitivity, as well as reported vision-related quality of life, were worse in patients than in controls, there were no significant changes in RNFL and macular thickness between the two groups. Moreover, no association was observed between RNFL thickness and disability as quantified with SARA score. Therefore, this study did not document retinal thinning as a prevalent or disease-specific finding in SCA14, suggesting that retinal atrophy does not contribute to the visual impairment in this cohort.

Retinal examination of patients with SCA, especially in those subtypes known to be associated with ophthalmic involvement is important in both clinical and research settings. OCT has been shown to have potential as a marker of disease progression in some SCAs, although given small sample sizes may be more relevant for group analysis rather than for individuals.

7.1.2 Episodic ataxias (EAs)

The EAs are rare neurological disorders characterised by recurrent episodes of cerebellar ataxia, often triggered by physical or emotional stress, infections or alcohol [245]. At present, there are at least seven known subtypes of EA. EA1 and EA2 are recognised as the most common [245] with EA2 being the third most frequent ataxia in the Sheffield Ataxia Centre, UK, after FRDA and SCA6 [25]. EA2 is caused by heterozygous mutations in the *CACNA1A* gene, which is linked to allelic AD conditions: hemiplegic migraine type 1 and SCA6.

The symptom onset in EA2 is typically in adolescence and EA2 episodes traditionally last between minutes and hours. These can be accompanied by migraine-like cephalgia in approximately 50% of patients [246]. However, none of the 42 patients with EA2 in the Sheffield Ataxia Centre had any clinical evidence of ataxia of an episodic nature, but instead had a slowly progressive ataxia often associated with cognitive impairment.

Eye movement disorders, including paroxysmal tonic upgaze, abnormal saccades, gaze-evoked or downbeat nystagmus have been reported both in adults and children with *CACNA1A* mutations [247, 248]. In the literature, data on retinal findings in individuals with EA are scarce. In one study which included children and adolescents, of 7 individuals with acetazolamide – responsive EA one had abnormal disc with pallor documented on fundoscopy [249]; in two sisters with biallelic *CACNA1A* pathogenic variants [250] optic nerve atrophy was also reported.

No studies to date have documented OCT findings in EA.

7.1.3 *SAMD9L*-associated ataxia pancytopenia syndrome

Complex inherited neurological disorders are a group of conditions that can be classified by the predominant neurological feature (e.g. cerebellar ataxia), type of neuropathy (e.g. demyelinating) and other associated features (neurological and non-neurological) [251]. Most commonly, the neuropathies seen in these complex inherited conditions are axonal. There are few conditions in which a neuropathy with slow conduction velocities is found as part of a complex neurological phenotype.

SAMD9L is a protein that is widely expressed across human tissues. It contains a SAM domain, allowing it to bind RNA and oligomerise with SAM-containing and non-SAM-containing proteins. The gene is located on chromosome 7q. The exact function of *SAMD9L* is unknown but it is thought to act as an anti-proliferative protein and has been shown to function as a tumor suppressor in breast, hepatocellular and squamous cell carcinoma [252]. Pathogenic variants in *SAMD9L* have been described in association with ataxia-pancytopenia (ATXPC) syndrome. ATXPC syndrome was first described by Frederik Li in 1978 [253]. He reported a family with neurological symptoms and pancytopenia. To date, only 38 patients with ATXPC syndrome have been reported in the literature [252 - 259].

7.2 Specific aims

As discussed in more detail in Chapter 2, the specific aims of this study were to describe patients with ADCA and complex AD syndromes that have ataxia as an associated feature; to characterise their retinal findings using OCT; to determine whether the pattern of retinal changes (when present) in patients with ADCA possesses any specificity. I also sought to determine if retinal changes detected by OCT are evident over time.

7.3 Methods

All affected individuals had clinical assessment using a standardised approach, comprising demographic information, history, pedigree, and detailed neurological examination as outlined in Chapter 4.

The Scale for the Assessment and Rating of Ataxia (SARA, range from 0-40 points with higher scores indicating more severe disease) [99] (Appendix 3) was obtained for each affected individual.

The best-corrected visual acuity (BCVA) was measured using Snellen chart and results were expressed in logarithm of the minimal angle of resolution (LogMAR) for analysis.

Each participant had a spectral-domain OCT examination with Topcon 3D OCT-2000 as described in details in Chapter 4.

Follow up assessment was performed in 2 individuals.

Descriptive statistics were used to summarise the characteristics of this group.

7.4 Results

7.4.1 Clinical phenotype and OCT findings in SCAs and EA

12 patients with genetically confirmed ADCA were included: SCA1 (n = 1), SCA2 (n = 1), SCA3 (n = 2), SCA6 (n = 1), SCA7 (n = 2), SCA11 (n = 1), SCA14 (n = 2), SCA17 (n = 1), EA2 (n = 1). Table 7.1 demonstrates demographics, disease duration, defined as the number of years since the first onset of gait instability and disease severity as quantified with SARA in the ADCA group.

Table 7.1 Demographic and disease characteristics in individuals with ADCA

Patient	Sex	Age at onset	Age at exam	Disease duration	SARA
SCA1.1	M	30	33	3	6.5
SCA2.1	F	22	42	20	22.5
SCA3.1	M	34	36	2	4.5
SCA3.2	F	40	54	14	28
SCA6.1	F	63	75	12	22
SCA7.1	M	39	46	7	7.5
SCA7.2	F	28	31	3	7.5
SCA11.1	F	29	37	8	8.5
SCA14.1	M	39	50	11	11
SCA14.2	F	35	47	12	3
SCA17.1	M	41	57	16	8
EA2	F	10	28	18	0.5

Age at onset, age at examination and disease duration are presented in years

Optical coherence tomography

Results of patients OCT studies were compared with healthy controls.

Sixty nine healthy controls of comparable age and sex were included. Mean age at assessment was 46 ± 13.6 years, range between 20 and 73 years. Summary of OCT findings in healthy controls is presented in Table 7.2.

Table 7.2 OCT findings in normal controls

				3D Macula		3D Disc				
	M / F ratio	Age (years)	BCVA (LogMAR)	Average (μm)	Foveal (μm)	Average RNFL (μm)	Superior (μm)	Nasal (μm)	Inferior (μm)	Temporal (μm)
mean $\pm$ SD	33 / 36	46 ± 13.6	0.05 ± 0.07	270.92 ± 12.7	240.3 ± 26.7	102.9 ± 7.6	119.9 ± 11.3	90.1 ± 12	127.2 ± 11.7	74.8 ± 8.7
range		(20 - 73)	(-0.1 to + 0.3)	(245 - 300)	(201.5 - 308.5)	(83 - 114)	(89 - 144)	(71 - 124)	(96 - 152)	(60 - 93)

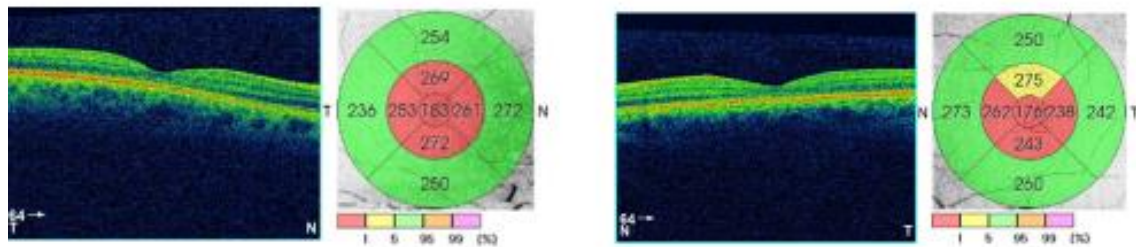
Results of the OCT studies of individuals with ADCA are summarised in Table 7.3. 3D optic disc scans from two individuals (SCA3.2 and SCA17.1) were excluded from analysis due to poor image quality (image quality score $\leq 50/100$).

Table 7.3 Visual acuity and OCT findings in ADCA patients

Patient	VA		Average macula thickness (μm)	Foveal thickness (μm)	Average RNFL thickness (μm)	Superior RNFL thickness (μm)	Nasal RNFL thickness (μm)	Inferior RNFL thickness (μm)	Temporal RNFL thickness (μm)
	R	L							
SCA1.1	20/100	20/20	265.3	191.5	105	109.5	82	150.5	78
SCA2.1	20/20	20/20	247.4	215.5	91.5	105	74.5	114	72.5
SCA3.1	20/20	20/20	290.6	239	91	112	87	128.5	74.5
SCA3.2	20/40	20/25	269	221.5	NA				
SCA6.1	20/100	20/40	257.9	180	103	113	91	122	85
SCA7.1	20/25	20/20	243.7	145.5	78	81	68.5	96.5	64.5
SCA7.2	FC	20/200	191	139	89.5	95.5	80.5	118	64
SCA11.1	20/20	20/20	267.15	269.5	94	113	82.5	124.5	55
SCA14.1	20/20	20/20	293.55	330	111	124	86.5	119	107
SCA14.2	20/20	20/20	294.4	245	107	131	99	124	73.5
SCA17.1	20/40	20/40	284.35	254	NA				
EA2	20/20	20/40	265.2	268	114.5	138.5	103	137	79.5

Among patients with SCA1, 2, 3 and 6, the average peripapillary, as well as sectorial RNFL thickness was within the normal range in all the individuals in whom 3D scans were obtained. When comparing the thickness in the macular region, the foveal thickness and the thickness in the inner macular ring was reduced in SCA1 and SCA6 (Figure 7.1), while the average macular thickness was reduced in the individuals with SCA2 and SCA6 in comparison to controls.

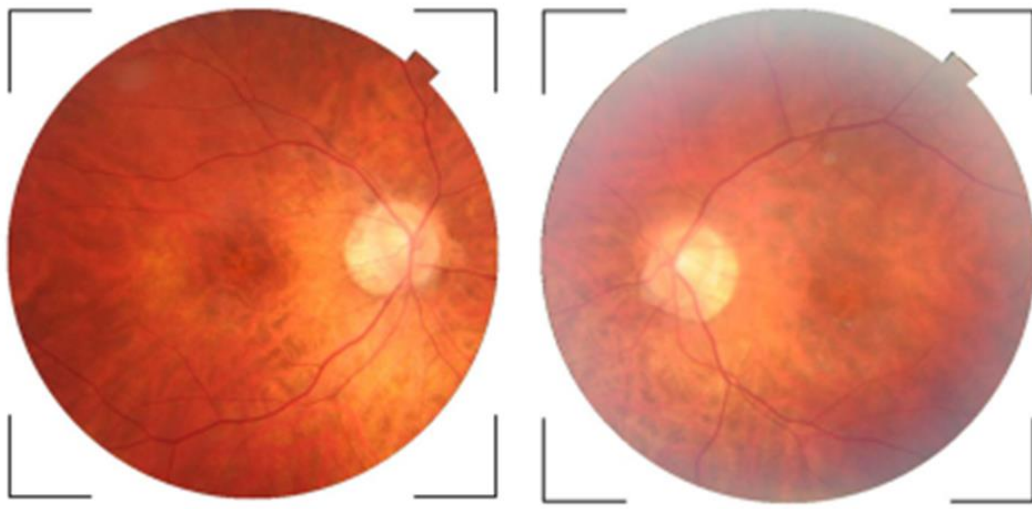
Figure 7.1 Macular appearance in SCA1



Macular 3D scan in patient SCA1.1 demonstrating reduced thickness in the fovea and the inner macula ring according to the ETDRS grid.

Fundus images of both individuals with SCA7 were abnormal with more marked changes in patient SCA7.2 (Figure 7.2), who developed poor vision since the age of 7 years and was legally blind by age of 24. Difficulty walking was first noticed at the age of 28 years. In contrast, patient SCA7.1 developed slowly progressive balance difficulty from 38 years of age and has no subjective visual complaints.

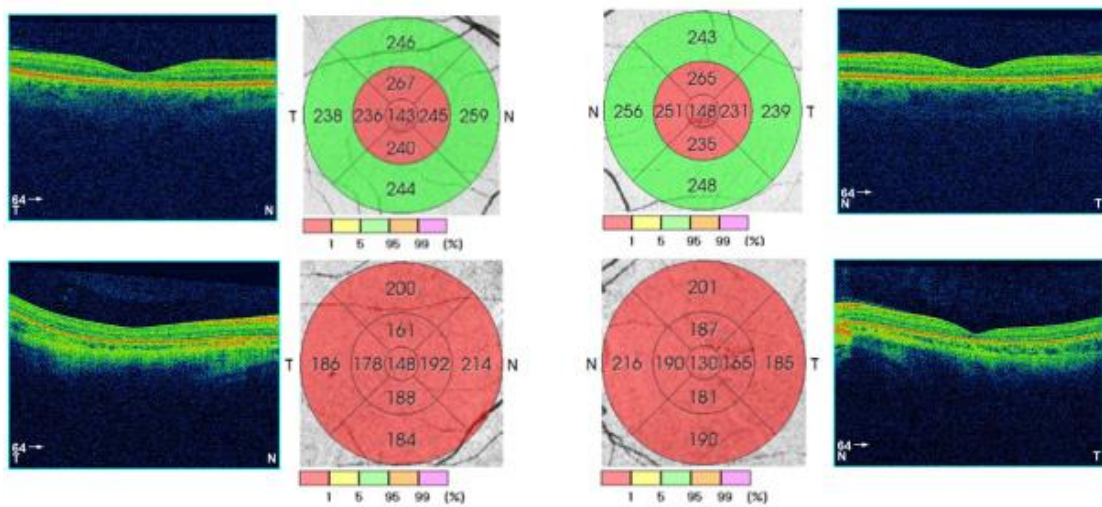
Figure 7.2 Fundus appearance in SCA7



Fundus image of patient SCA7.2 showing pigment changes in the fovea, diffuse areas of hypopigmentation in the peripheral retina and vascular attenuation

Macular OCT analysis in both SCA7 patients showed a noticeable degree of foveal thinning (Table 7.3, Figure 7.3) with mean foveal thickness 142.25 ± 4.6 which is considerably different from controls (mean 240.3 ± 26.7). The average macular thickness in SCA7.1 and SCA7.2 was the lowest among individuals with ADCA (mean 217.35 ± 37.3) and visibly reduced when compared to healthy controls (mean 270.92 ± 12.7).

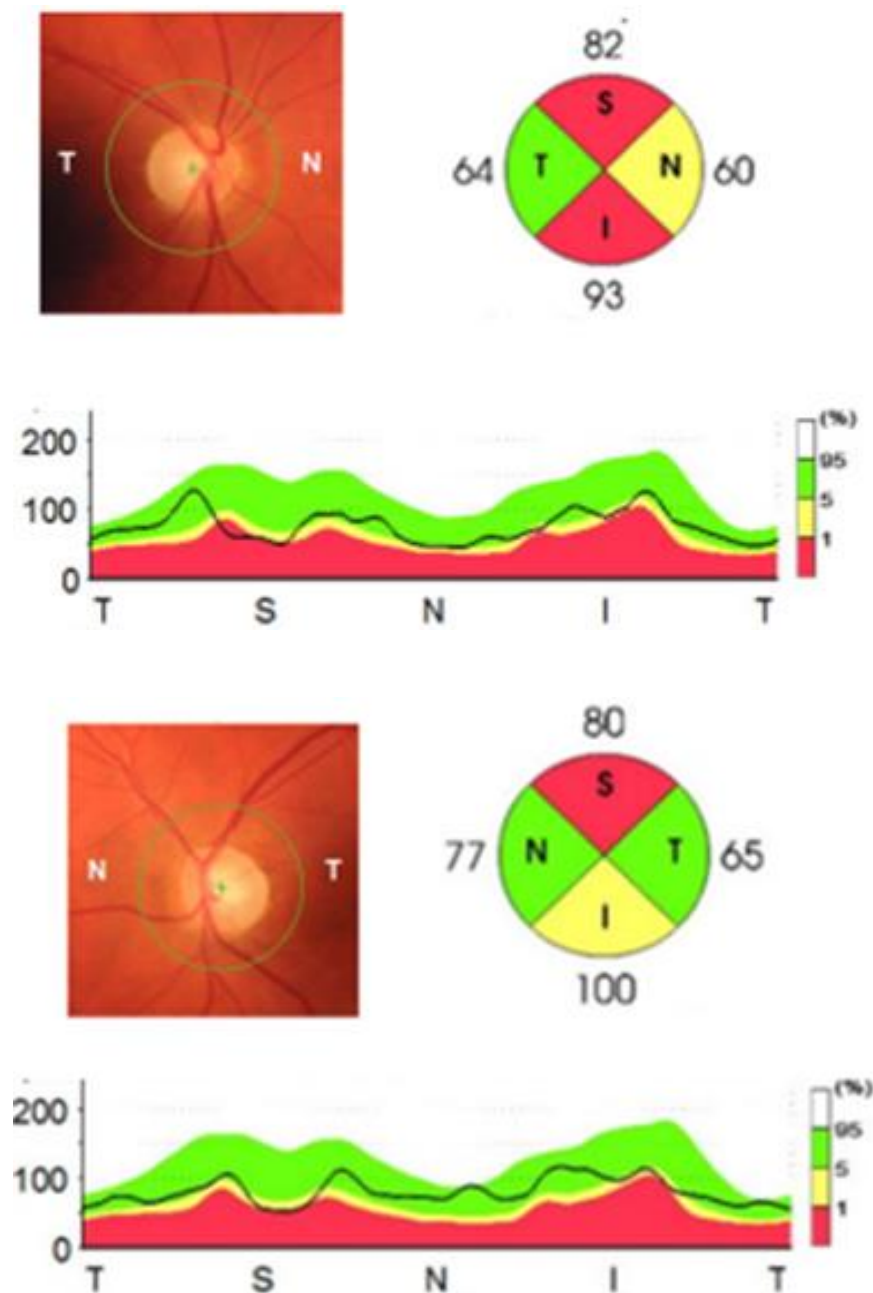
Figure 7.3 Macular appearance in SCA7



Macular 3D scan in patient SCA7.1 (top) demonstrating reduced thickness in the fovea and the inner macula ring according to the ETDRS grid. In patient SCA7.2 (bottom) there is also abnormal thinning in the outer macular ring

The average peripapillary RNFL thickness in patient SCA7.1 was reduced (Figure 7.4). Analysis of OCT measurements by quadrant showed predominant abnormal RNFL thinning in the superior and inferior quadrants, while SCA7.2 had a tendency to reduced RNFL in the superior quadrant. Temporal RNFL thickness measurements in the SCA7 patients were among the three lowest in the ADCA group.

Figure 7.4 Optical coherence tomography of a patient with SCA7



OCT appearance in SCA7.1 (both eyes, right at the top, left at the bottom) showing optic nerve photograph with peripapillary ring scan indicated with green circle; pie graph of quadrants with RNFL thickness and RNFL circular tomogram representing quantitative analysis of RNFL thickness (black line) and normative data set (green area = 95% confidence interval, yellow area = 99% CI, red area = outside 99% CI). Quadrants: T = temporal; S = superior; N = nasal; I = inferior

Although the average peripapillary RNFL thickness in the SCA11 patient was within the normal range, there was RNFL reduction in the temporal sector when compared with other ADCA cases and healthy controls. No abnormal macular thickness was observed.

The average peripapillary, as well as sectorial RNFL thickness was within the normal range in the two individuals with SCA14. Average macular and foveal thickness were also normal. Similarly, no abnormal thinning was observed in the macular and optic disc areas in the EA2 patient.

Only macular scans with good image quality were obtained from the individual with SCA17 and analysis did not reveal any abnormal thickness.

Longitudinal assessment

Patients SCA1.1 and SCA 3.1 had repeat OCT studies 29 and 18 months after initial assessment respectively.

Table 7.4 shows the mean measurements from both eyes of each patient at initial and follow-up assessments. There was a tendency for average macular and RNFL thickness reduction in SCA1.1, but not in SCA3.1. Similarly, noticeable disability progression as quantified with SARA score was observed in SCA1.1 with subtle advancement in the SCA3.1 individual.

Table 7.4 BCVA, SARA score and OCT results in patients with SCA1 and SCA3 at baseline and at follow-up

SCA1.1	VA R L	SARA	Average macula thickness (μm)	Foveal thickness (μm)	Average RNFL thickness (μm)	Superior RNFL thickness (μm)	Nasal RNFL thickness (μm)	Inferior RNFL thickness (μm)	Temporal RNFL thickness (μm)
Baseline (33 yr)	20/100 20/20	6.5	265.3	186	105	109.5	82	150.5	78
Follow-up (36 yr)	20/100 20/20	9	263.5	183	102	109.5	80	147	75
SCA3.1	VA R L	SARA	Average macula thickness (μm)	Foveal thickness (μm)	Average RNFL thickness (μm)	Superior RNFL thickness (μm)	Nasal RNFL thickness (μm)	Inferior RNFL thickness (μm)	Temporal RNFL thickness (μm)
Baseline (36 yr)	20/20 20/20	4.5	290.6	239	100	112	87	128.5	75
Follow-up (37 yr)	20/20 20/20	5	291.2	241	100	111.5	86.5	127	75.5

Genetic analysis

Patients with SCA1, 2, 3, 6, 7 and 17 were diagnosed following repeat expansion disorder testing. Diagnosis was obtained through NGS panel testing in SCA11 and EA2 patients, while WES was diagnostic in SCA14.1; SCA14.2 patient was tested directly for the known familial variant.

7.4.2 Clinical phenotype and OCT findings in *SAMD9L*-associated ataxia pancytopenia syndrome

A 66-year-old lady had a normal birth and neonatal period. Developmental milestones were normal. She had always been aware of high-arched feet. She reported poor balance, tripping easily in the first decade and, at 12 years of age, sought attention because she found it hard to keep shoes on her feet. At age 16, she was diagnosed with Charcot-Marie-Tooth disease (CMT); in adulthood this was further classified as CMT type 1 based on neurophysiological testing. She remained stable until her mid-50s, being independently mobile with normal hand function. In her mid-50s, her gait became progressively more unsteady, initially requiring a stick but progressing to a rollator within 6 years and being wheelchair dependent in her 60s. She also began to develop

dysarthria and dysphagia, requiring a modified diet. She is one of 8 siblings in a family with no consanguinity and no family history of any neurological impairment or haematological malignancy.

On examination, she had prominent conjunctival blood vessels with tortuosity and telangiectasia, moderate-to-marked midline ataxia, bilateral pes cavus, downbeating nystagmus, cerebellar dysarthria, brisk reflexes and extensor plantars. Vibration and pinprick were reduced distally. She was only able to take a few steps with bilateral support.

Nerve conduction studies

Nerve conduction studies demonstrated a length-dependent sensorimotor neuropathy with prolonged distal motor latencies and reduced conduction velocities within the demyelinating range (Table 7.5).

Table 7.5 Nerve conduction studies in a patient with *SAMD9L*-associated ataxia pancytopenia syndrome

Motor Nerve Conduction Studies			
Site	Latency (ms)	Amplitude (mV)	NCV (m/s)
Median, L			
Wrist	4.41	9.4	
Elbow	11.1	6.5	29.9
Ulnar, L			
Wrist	3.48	7.1	
Below Elbow	10.35	7.1	24.7
Median, R			
Wrist	5.59	7.6	
Elbow	11.31	7.1	36.1
Ulnar, R			
Wrist	3.6	10.4	
Below Elbow	9.48	7.2	30.6
Tibial, R			
Ankle	7.1	1.19	
Peroneal, R			
Ankle	7.2	0.11	
Below knee	18.9	0.09	31.6
Sensory Nerve Conduction Studies			
Site	Latency (ms)	Amplitude (uV)	NCV (m/s)
Median, R			
Digit I – Wrist	3.38	7.25	29.6
Ulnar, R			
Digit V – Wrist	3.3	5.9	27.3
Radial, R			
Forearm	2.52	15.9	31.7
Sural, R			
Mid-calf	Absent	Absent	Absent

mV=millivolts; uV=microvolts; ms=milliseconds; m/s=metres per second; R=right; L=left. Abnormal values in **bold**

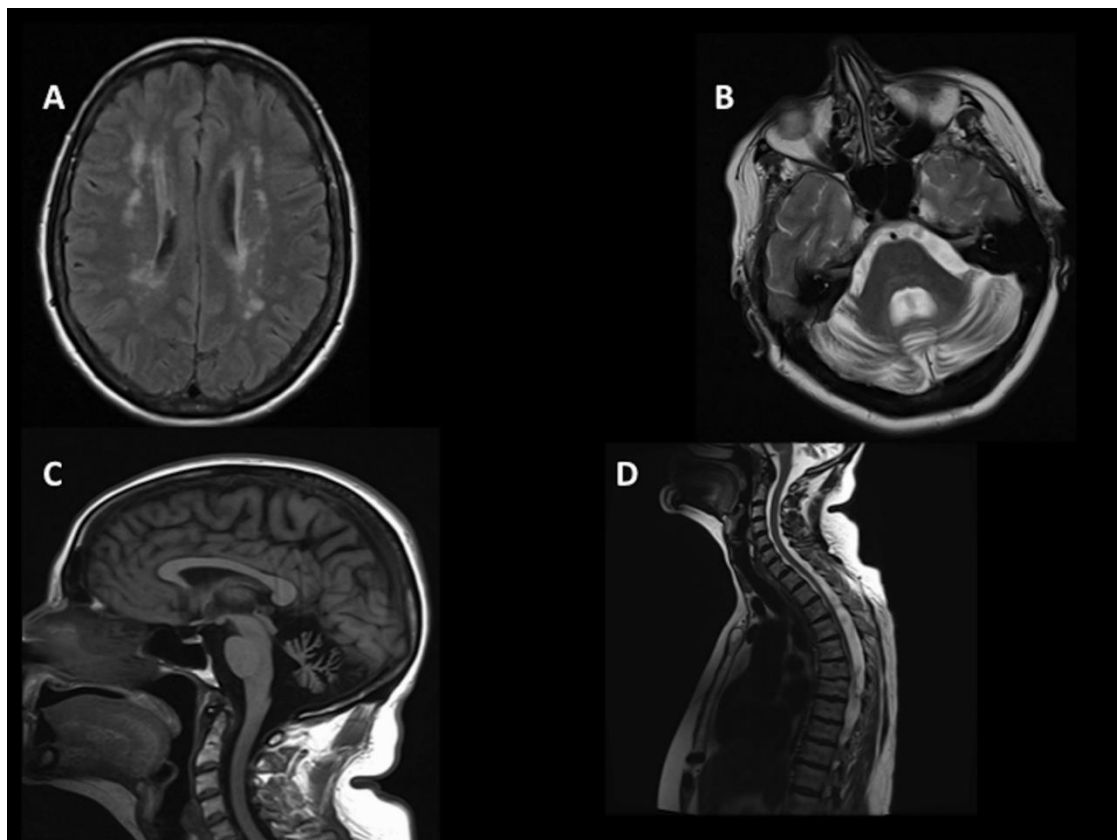
Normative values are shown in Appendix 8

Routine laboratory investigations (full blood count, renal and liver profiles) did not show any abnormalities.

Brain MRI

MRI brain showed marked cerebellar atrophy as well as multiple foci of high signal in the deep white matter of the cerebral hemispheres bilaterally. There was also T2 hyperintense signal in the cerebellar peduncles. MRI spine showed volume loss in the thoracic cord (Figure 7.5).

Figure 7.5 MRI imaging of patient with *SAMD9L*-associated ataxia pancytopenia syndrome

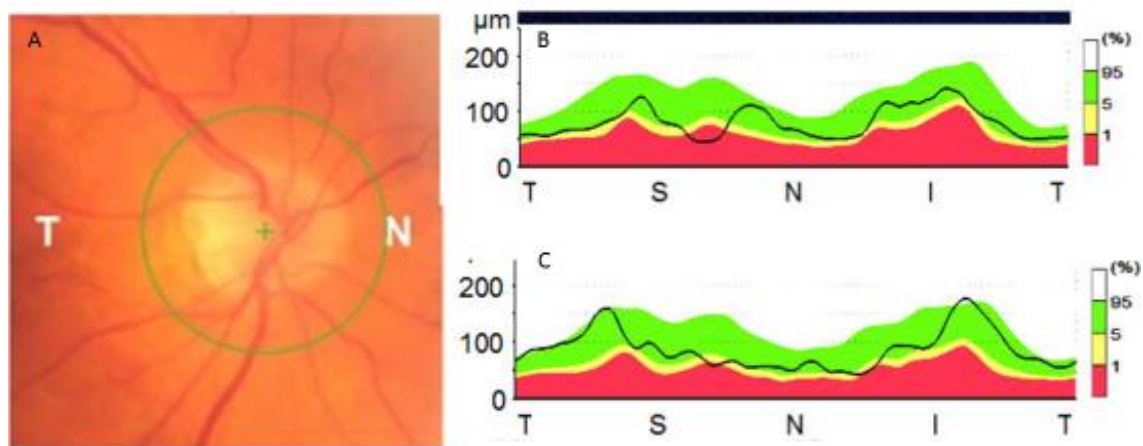


Axial FLAIR sequence showing multiple bilateral foci of high signal in the deep white matter of the cerebral hemispheres (A). Axial T2 sequence (B) demonstrating increased signal in the cerebellar peduncles. Sagittal T1 (C) sequence showing marked cerebellar atrophy. Sagittal T2 sequence from MRI spine showing volume loss in the thoracic cord (D).

Optical coherence tomography

Optical coherence tomography (OCT) showed global thinning of the retinal nerve fibre layer (Figure 7.6).

Figure 7.6 Optical coherence tomography of the patient with *SAMD9L* associated ataxia pancytopenia syndrome



A. OCT appearance of the right eye. B. Global right eye RNFL thinning, 84 μm. RNFL thickness (black line) demonstrates predominantly superior nasal RNFL loss. C. Global left eye RNFL thinning, 88 μm with predominantly superior nasal RNFL thinning. (Quadrants: T = temporal; S = superior; N = nasal; I = inferior)

Genetic results

PMP22 dosage analysis performed before onset of ataxia was normal. Genetic testing was negative for FRDA, SCA 3 and SCA6 and autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS). A next generation sequencing panel of 98 genes associated with ataxia did not reveal any causative variants. In addition, a next generation sequencing panel of 277 genes associated with neuromuscular disorders including Charcot-Marie-Tooth disease did not identify any relevant variants.

Diagnostic laboratory whole-exome sequencing (WES) showed a c.2956C>T p.(Arg986Cys) variant in the *SAMD9L* gene (Table 7.6). This is a missense variant affecting a highly conserved amino acid and is not seen at any allele frequency in available genome databases (1000 Genomes, Exome Sequencing Project, CentoMD, Genome Aggregation Database). This variant has been reported previously as pathogenic by several authors [256, 259]. Both her parents are deceased and there are no living affected relatives for segregation analysis of this variant.

Table 7.6 WES performed by Centogene – result summary

Gene	Transcript	cDNA change	AA Change	Zygosity	In silico parameters*	Allele frequencies	Type and classification ***
<i>SAMD9L</i>	NM_0013033496.1	c.2956C>T	p.(Arg986Cys)	Het	<u>Polyphen</u> : Benign <u>Align-GVGD</u> : C0 SIFT: Deleterious <u>Mutation Taster</u> : Disease causing <u>Conservation nt</u> : moderate <u>Conservation aa</u> : high	gnomAD: - ESP: - 1000 G: - CentoMD: -	Missense Pathogenic (class 1)

*AlignGVD: C0 least likely to interfere with function, C65 most likely to interfere with function, splice prediction tools: SSF, MaxEnt, HSF

**Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available)

***Based on ACMG recommendations

7.5 Discussion

This study investigated the retinal involvement in patients with ADCA and complex autosomal dominant disorder associated with ataxia (*SAMD9L* - associated ataxia pancytopenia syndrome) using OCT.

Individuals with SCA7 had most prominent afferent visual system involvement, as seen in previous SCA7 studies [111]. As expected, the two individuals in this study had different degree of maculopathy with foveal atrophy present in both. In contrast to larger studies, where normal average RNFL thickness has been documented in most patients [115], thinning of the RNFL was seen in this study with predominant changes in the superior more than inferior quadrant. Visual impairment due to macular deterioration has been well documented in SCA7 cohorts and in accordance with the literature, significant visual loss was seen in one of the SCA7 patients in this study, which preceded the onset of ataxia. It is unclear why in some subjects the disease begins with visual loss and in others with gait difficulty. It would be interesting to monitor these two individuals who had different initial manifestations with visual loss in one and gait ataxia in the other, over time, and see if retinal and cerebellar presentations progress with similar rates. Longitudinal studies will be very helpful to further clarify the usefulness of OCT in this subgroup of patients and might have implications in future therapeutic trials.

In contrast to prior studies where reduced overall RNFL thickness has been observed in SCA1, SCA2 and SCA3 [38, 105, 106, 109], in this study the average peripapillary, as well as sectorial RNFL thickness was within the normal range. Similarly to the mixed SCA study, the overall thickness in the macular region was thinner in the individuals with SCA1 and SCA6 [38], but in contrast to the larger study, reduced macular thickness was found in SCA2. In patients with SCA1, who have normal appearance of the macula, and the optic disc on fundoscopy, macular degeneration as detected with OCT has been

well described and recognised as an important cause of visual loss [260]. However, the literature is controversial, as some reports suggest only peripapillary RNFL and not macular thinning in SCA1 [105]. In contrast to the latter, the SCA1 patient here not only displayed macular thinning, but also a tendency to further thinning over time.

Interestingly, in this study the individual with SCA11 had some temporal RNFL thinning, similarly to the *SPG7* cohort, discussed in detail in Chapter 6. SCA11 is a rare cause of SCA in Caucasians, accounting for less than 1% of dominant ataxias in central Europe [261]. Retinal changes as detected by OCT have not been previously reported. The SCA11 patient in this study presented with a spastic-ataxic phenotype, had normal vision and funduscopy appearance. Although the average peripapillary RNFL thickness was normal, there was reduction in the temporal RNFL thickness in comparison to other ADCA and healthy controls, similarly to previously described cases with complex HSP-phenotypes [130, 212].

This study confirmed previously reported normal retinal findings in patients with SCA14 [120] and further supports the concept that, in contrast to other SCAs, maculopathy and RNFL thinning are not prevalent or disease-specific for this entity.

The patient with childhood onset of demyelinating neuropathy, initially diagnosed as CMT1, which remained clinically stable until development of a progressive cerebellar ataxia more than 40 years later was found to have a pathogenic variant in *SAMD9L*. This c.2956C>T p.(Arg986Cys) variant has previously been described in association with ataxia-pancytopenia (ATXPC) syndrome in a large multigenerational family, only one of whom was reported to have neuropathy [256, 259]. The neurological phenotype in ATXPC syndrome is variable, even within families. Almost all carriers of pathogenic *SAMD9L* variants have some neurologic involvement. Balance problems and nystagmus

are the most common features, followed by mild pyramidal signs such as brisk reflexes, spasticity and ankle clonus. Some patients displayed dysmetria. Two patients reported difficulties with gaze fixation and reading and underwent multifocal electroretinography, which showed paracentral retinal dysfunction [259]. Only seven patients have been reported in the literature to have neuropathy as part of the clinical phenotype; conduction velocities were reported as “low normal” in one case [254] and “demyelinating” in another [258] but nerve conduction data is not available for the other cases. There are no previous reports of a prolonged period of stability followed by a later deterioration such as seen in this case. Typical MRI findings include cerebellar atrophy and periventricular white matter hyperintensities [252]. Thinning of the spinal cord has been described in one previously reported patient [258]. Cerebellar peduncle changes are not previously described. The degree of cerebellar atrophy does not appear to correlate with the severity of balance impairment. One patient was reported to have very mild balance impairment despite very pronounced atrophy [258]. Autopsy examination of deceased patients has shown cerebellar and inferior olivary atrophy with severe loss of Purkinje cells [253, 255]. In one patient, white matter myelination was normal but there was extensive white matter gliosis in the hippocampus [255]. The exact neuropathological cause of these white matter lesions is not known but it has been hypothesised that it is the result of slowing of normal physiological myelination in childhood and adolescence [252].

Haematological features also vary widely. The most common finding is cytopaenia, which can be uni-, bi- or trilinear but these are often transient [255, 256, 258]. Cytopaenias ranged from mild to severe with one thrombocytopaenic patient dying from gastrointestinal haemorrhage at age 79 [255] and another from non-traumatic intracranial haemorrhage at age 6 [257]. Aplastic anaemia has commonly been reported

as well as neutropaenia associated with chronic infection [255]. Three patients have been identified who developed myelodysplastic syndrome with an inherited *SAMD9L* pathogenic variant [256]. Another patient with a pathogenic variant in this gene developed acute lymphoblastic leukaemia (ALL) [257].

ATXPC syndrome is caused by a gain of function pathogenic variant in *SAMD9L*. It increases the normal suppressing effect of the *SAMD9L* protein on precursor cell divisions. In the patients with myelodysplastic syndrome, there was loss of chromosome 7q, through monosomy 7, derivative chromosome (1;7) and deletion of 7q, leading to loss of the suppression on cell proliferation through loss of the genetic material on chromosome 7 [255, 256]. The patient who developed ALL was found to have a pathogenic *SAMD9L* variant as well as a t(12;21) translocation, the most common translocation observed in childhood ALL [257].

Our patient had a history of a non-progressive demyelinating neuropathy with decades of stability before developing a progressive ataxic syndrome. She was not found to have any haematological abnormalities. However, cytopenias in this condition can be transient so it is possible that she was cytopenic at times between blood tests.

The differential diagnosis for inherited demyelinating neuropathies is limited [251]. This patient had clear slowing of conduction velocities on nerve conduction studies, as has been reported in the two prior cases [254, 258] suggesting an association between ATXPC syndrome and demyelinating neuropathy.

This is the first description of the retinal findings using OCT in ATXPC. This patient was found to have not only abnormal global RNFL thinning, but also predominant superior nasal RNFL loss, suggesting that *SAMD9L*-related phenotype might be associated with specific RNFL pattern. However, as these findings were observed in a

single patient, further OCT studies in complex AD ataxic syndromes are needed to see if these results can be replicated.

In the heterogeneous group of ADCA among the 10 individuals who had RNFL thickness assessment only one of the two individuals with SCA7 had reduced global RNFL thickness. In contrast, statistically significant global RNFL thickness reduction in comparison to controls has been previously documented in SCA1 [106], SCA2, and SCA3 [38, 109], while SCA7 is associated with more widespread effect on the visual system [113 - 115].

7.6 Conclusions

These data support the importance of obtaining OCT in all patients with ADCA, and particularly in those with reduced vision, as macular pathology and subtle optic neuropathy cannot always be reliably detected on funduscopy examination.

The retinal findings in the patient with *SAMD9L*-associated ataxia pancytopenia syndrome, previously not described and extending the already reported phenotype to include RNFL thinning, suggest that OCT may be a useful additional tool in distinguishing this complex AD disorder associated with ataxia from other ataxic syndromes without retinal involvement; however further studies are needed to see if these results could be replicated.

Chapter 8: Clinical phenotype and optical coherence tomography in autosomal recessive spastic ataxia of Charlevoix-Saguenay

8.1 Introduction

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a rare neurodegenerative disorder typically characterised by an early-onset cerebellar ataxia, slowly progressive lower limb spasticity and axonal-demyelinating sensorimotor peripheral neuropathy [121]. ARSACS was first described in individuals from the Charlevoix and Saguenay-Lac Saint Jean regions of North-Eastern Québec, but cases have been described subsequently in Europe, North Africa, and Brazil among the others. ARSACS is caused by mutations in the *SACS* (Sacsin Molecular Chaperone) gene located on chromosome 13q12.12 [122]. The *SACS* gene encodes sacsin, a protein most highly expressed in cerebellar Purkinje cells, thalamic, midbrain, precerebellar and brainstem nuclei, and in the large pyramidal forebrain neurones [123]. The protein is thought to have a role in the regulation of mitochondrial dynamics, leading to mitochondrial dysfunction [124].

Thickening of RNFL, which appears as prominent streaks in all directions from the optic disc, most noticeable in the papillomacular bundle area, is the characteristic retinal change typically seen on fundoscopy in patients with ARSACS [125]. However, these retinal changes are not consistently observed in non-Québécois cases.

In the last decade, OCT has demonstrated to be a useful and sensitive method of detecting RNFL changes in ARSACS. Originally, a small number of patients have been shown to have a marked global thickening of the peripapillary RNFL with loss of the

foveal depression - features not seen in healthy individuals [126, 127]. Subsequently, Parkinson et al. characterised the OCT findings in a cohort of 17 patients with ARSACS and 13 asymptomatic heterozygous ARSACS carriers [91]. They demonstrated that although only 70% of affected individuals had peripapillary retinal striations visible on fundoscopy, all had thickening of the RNFL on OCT, a finding not observed in the study controls and other genetic ataxia cases. Peripapillary RNFL thickening as detected by OCT was also documented by Filho et al. in all 13 affected ARSACS Brazilian patients, of whom 2 had normal fundoscopy [128].

Thus, as these retinal findings appear to be sensitive biomarkers of ARSACS disease, routine use of OCT technique in the assessment of all suspected cases, even in the absence of funduscopy changes, has been proposed.

8.2 Specific aims

As discussed in more detail in Chapter 2, the specific aims of this study were to describe patients with recessive *SACS* variants; to characterise their retinal findings using OCT; to determine whether the pattern of retinal changes in patients with *ARSACS* possesses any specificity. I also sought to determine if retinal changes detected by OCT are evident over time.

8.3 Methods

All affected individuals had clinical assessment using a standardised approach, comprising demographic information, history, pedigree, and detailed neurological examination as outlined in Chapter 4.

The Scale for the Assessment and Rating of Ataxia (SARA, range from 0-40 points with higher scores indicating more severe disease) [99] (Appendix 3) was obtained for each affected individual.

The best-corrected visual acuity (BCVA) was measured using Snellen chart and results were expressed in logarithm of the minimal angle of resolution (LogMAR) for analysis.

Each participant had a spectral-domain OCT examination with Topcon 3D OCT-2000 as described in details in Chapter 4.

Follow up assessment was performed in 1 individual.

Descriptive statistics were used to summarise the characteristics of this group.

8.4 Results

Cases description

Three unrelated Caucasian symptomatic individuals (one male, one of Italian descent) with biallelic *SACS* variants, were included (Table 8.1). Consanguinity was reported in one family (patient 2). Mean age at assessment was 33 years. Disease duration varied between 15 and 49 years.

In all three the presenting symptom was gait disturbance with onset in the first decade of life (ages 2 to 5 years). Lower limb spasticity was universally present, while truncal and appendicular cerebellar ataxia and peripheral neuropathy were seen in patients 1 & 2, but not in patient 3. All patients had horizontal nystagmus, while saccadic pursuits were observed in patients 1 & 2. One individual (patient 2) had pes cavus and borderline IQ. Detailed characteristics are shown in Table 8.1.

All individuals had neurophysiology, which demonstrated length-dependent mixed type sensorimotor neuropathy in patients 1 & 2.

Table 8.1 Characteristics of individuals with biallelic SACS variants

Patient	Pt 1	Pt 2	Pt 3
SACS variants	c.[10907G>A; 10954C>A]		c.11380G>C
Nucleotide change	c.4453G>A	Hom c.3328dup	c.2825C>T
Amino acid change	p. [Arg3636Gln; Pro3652Thr] p.(Ala1485Thr)	Hom p.ile1110fs	p.Val3794Leu p.Thr942Ile
ACMG criteria	pathogenic likely pathogenic	pathogenic	VUS VUS
Sex	M	F	F
Age at onset	2 yr	2 yr	5 yr
Age at exam	27 yr	17 yr	56 yr
Disease duration	25 yr	15 yr	49 yr
EOM	Saccadic pursuits, horizontal GEN, upbeat Ny on upgaze	Saccadic pursuits, horizontal GEN	Subtle horizontal GEN
Dysarthria	+	+	-
Gait ataxia	++	++	-
Limb ataxia UL / LL	+ / ++	+ / ++	-
LL weakness	Distal	Proximal and distal	Proximal and distal
Spasticity LL	+	++	++
Reflexes LL	KJ +++ / AJ ++	KJ +++, AJ absent	KJ +++ / AJ +++
Plantar responses	↑↑	↑↑	↑↑
Other	-	Pes cavus; Cognitive impairment	-
Sensory loss	Vibration to knees	Vibration to cm	No
Neuropathy (NCS)	Yes	Yes	No
MRI Brain / Age	Moderate superior vermis atrophy; pontine T2 hypointensitis; ACC post 1/3 / 27 years	Mild superior vermis atrophy; pontine T2 hypointensitis / 16 years	Mild pancerebellar atrophy; ACC post 1/3 / 55 years
SARA	12	9.5	15

GEN, Gaze- evoked nystagmus; EOM, Extraocular movements; ACMG, American College of Medical Genetics guidelines; KJ, knee jerks; AJ, ankle jerks; NCS, Nerve Conduction Studies; SARA, Scale for the Assessment and Rating of Ataxia; + Mild, ++ Moderate, +++ Marked

Genetic analysis

All patients underwent next-generation sequencing (NGS) targeted panel testing, followed by whole-genome sequencing (WGS) in patient 3.

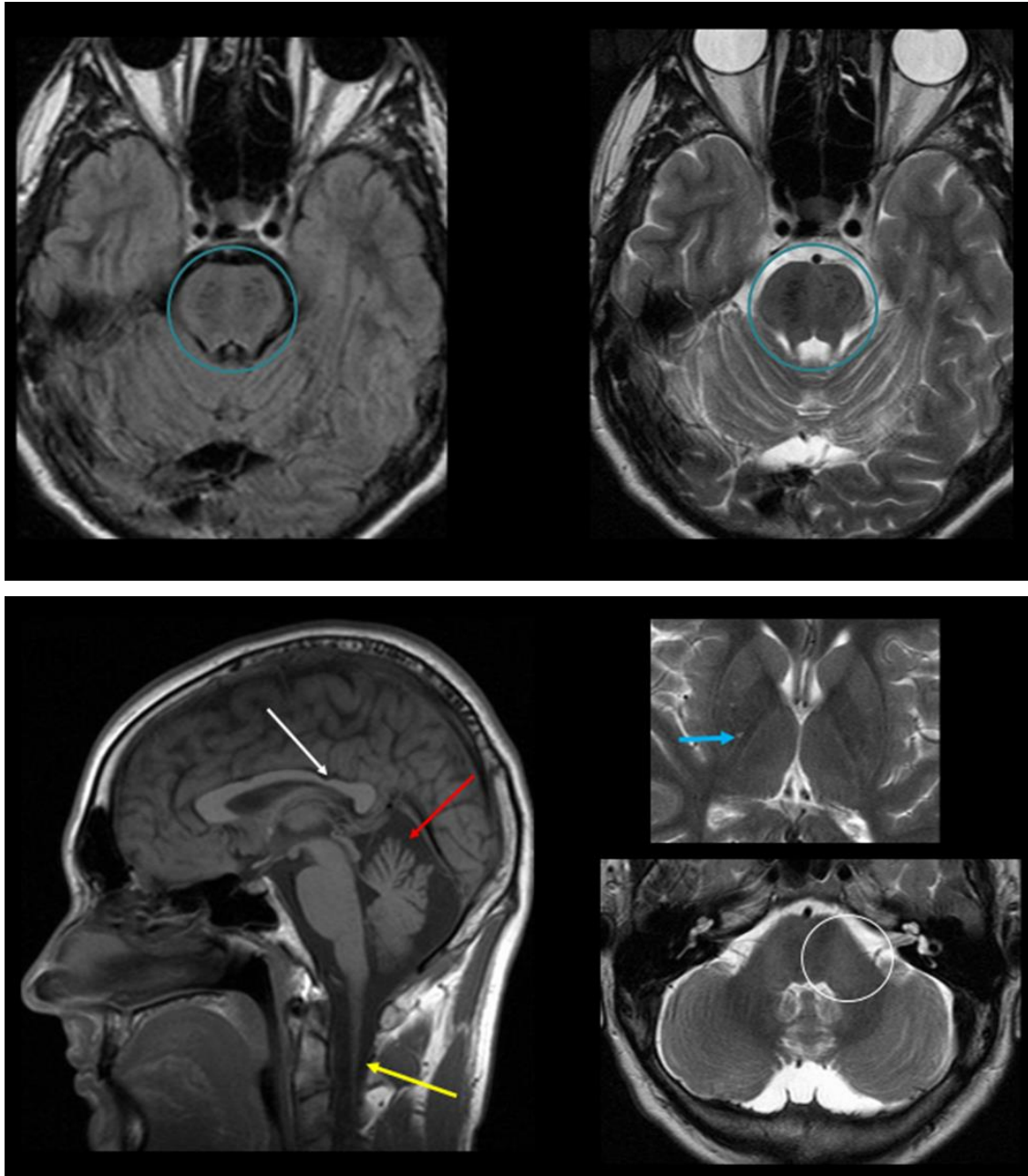
Of the five variants identified in our patients, two were previously reported pathogenic: c.3328dup (p.Ile1110fs) and c.[10907G>A; 10954C>A] (p. [Arg3636Gln; Pro3652Thr]. Patient 1 had a novel missense variant, c.4453G>A (p.Ala1485Thr), considered likely pathogenic according to American College of Medical Genetics (ACMG) guidelines.

Patient 3 had two previously not described missense SACS variants, classified as VUS when ACMG guidelines were applied. As these two variants are in compound heterozygosity, confirmed with parental testing, the diagnosis of ARSACS is possible, although her phenotype, consistent with uncomplicated spastic paraplegia, differs considerably from patients 1 and 2.

Brain MRI

Brain MRI showed cerebellar atrophy in all three, pancerebellar in patient 3 and vermis - predominant in patients 1&2, who also displayed linear pontine hypointensities (Figure 8.1).

Figure 8.1 Brain magnetic resonance imaging of patient with ARSACS

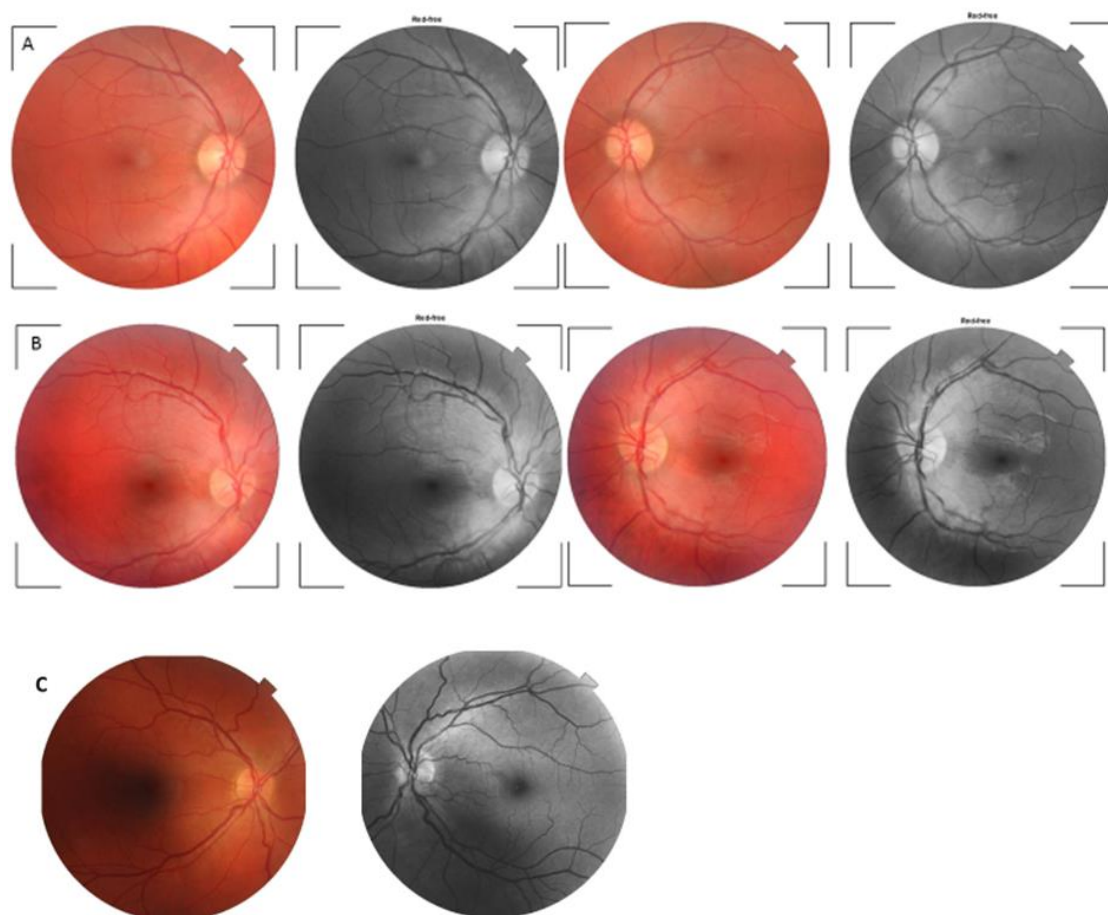


Radiological findings in patient 1 demonstrating transverse pontine hypointensities on FLAIR and T2- weighted imaging (top); superior vermian atrophy (red arrow), atrophy of posterior 1/3 of corpus callosum (white arrow), cervical cord atrophy (yellow arrow), perithalamic hyperintensity (blue arrow) and hyperintensity of the lateral pons at the level of the middle cerebellar peduncles (white circle)

Ophthalmological findings

None of the patients had visual complaints. Patient 1 had mild divergent strabismus. Average Snellen VA was 20/25 (range 20/20 to 20/40). Retinal pigmentation was not evident in any of the three individuals. Retinal thickening on fundoscopy was present bilaterally in patients 1&2 (Figure 8.2).

Figure 8.2 Fundus images of patients with ARSACS



Fundus imaging (retinography and red-free fundus images) showing thickening of the RNFL in Patient 1 (A) and Patient 2 (B). Visible streaks emanating from the optic disc are observed, most prominent in patient 1. In some places RNFL thickening obscures the normally sharp edges of the retinal vessels. C. Fundus imaging of a healthy control

Optical coherence tomography

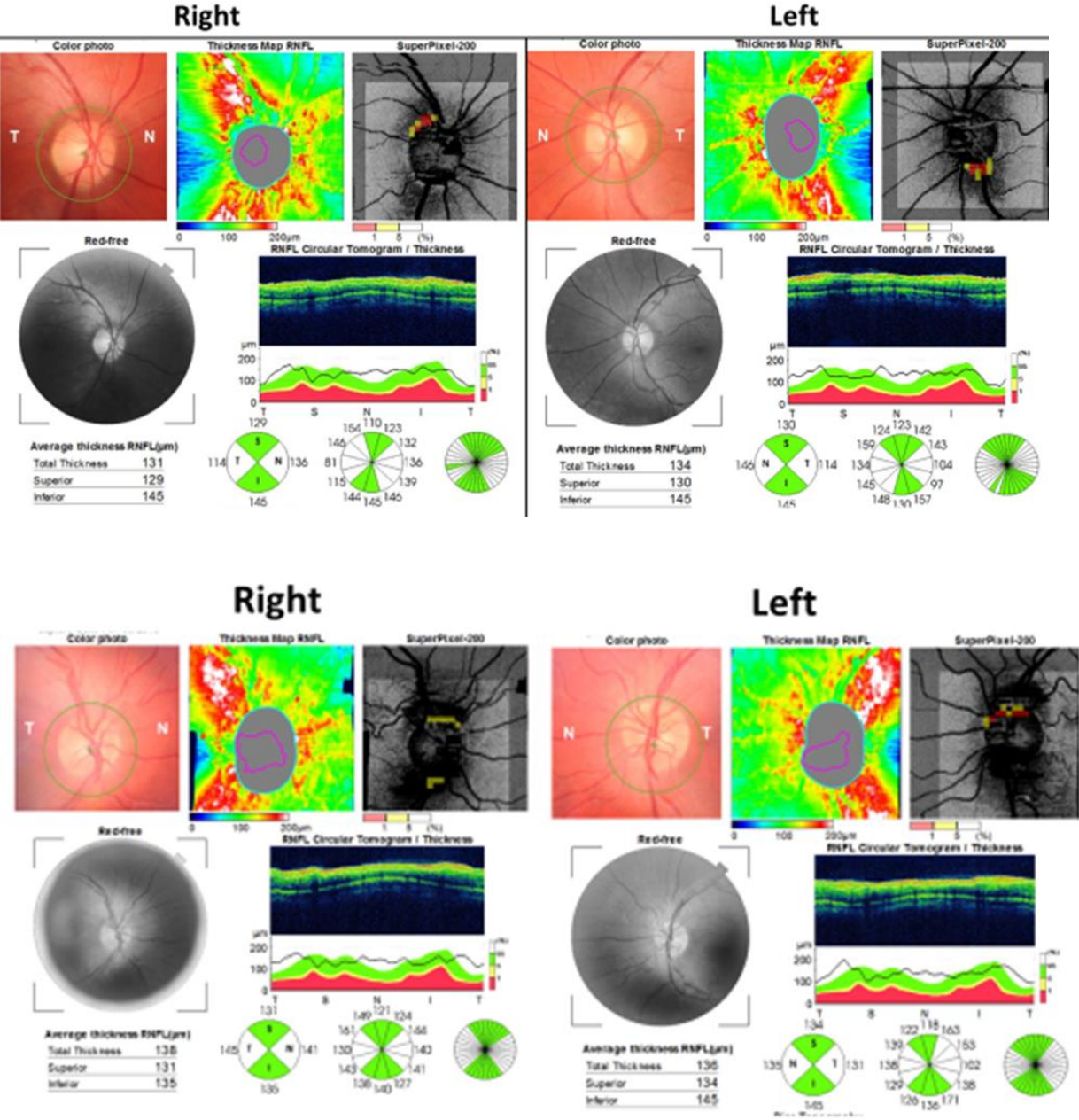
The results of the OCT studies are presented in Table 8.2. The average peripapillary RNFL thickness in patients 1 & 2 was above 95% upper limit of normal, defined in the normative database provided by the manufacturer. None of these patients had any associated swelling or elevation of the optic nerve head. Sectorial analysis of OCT measurements in both patients with ARSACS showed abnormal thickening of the RNFL predominantly in the nasal and temporal quadrants (Figure 8.3). In contrast to patients 1 & 2, where RNFL was visibly thickened, OCT measurements of patient 3 were much lower and comparable to those from healthy controls.

Table 8.2 Ophthalmological findings and RNFL thickness measured by OCT in patients with biallelic SACS variants

Patient	Pt 1	Pt 2	Pt 3
Visual acuity R	20/40	20/25	20/20
Visual acuity L	20/30	20/25	20/20
Fundoscopy	RT	RT	N
Average macula thickness (μm)			
OD	300.6	358.2	252.1
OS	296.6	362.9	252
Foveal thickness (μm)			
OD	307	344	217
OS	305	310	228
Average RNFL thickness (μm)			
OD	131	142	97
OS	134	136	86
Superior RNFL thickness (μm)			
OD	129	137	136
OS	130	134	107
Nasal RNFL thickness (μm)			
OD	147	136	97
OS	135	146	84
Inferior RNFL thickness (μm)			
OD	145	144	108
OS	145	145	108
Temporal RNFL thickness (μm)			
OD	114	142	74
OS	114	131	57

Visual acuity (Snellen), presented as best-corrected visual acuity; OD, right eye; OS, left eye; RT, retinal thickening; N, normal

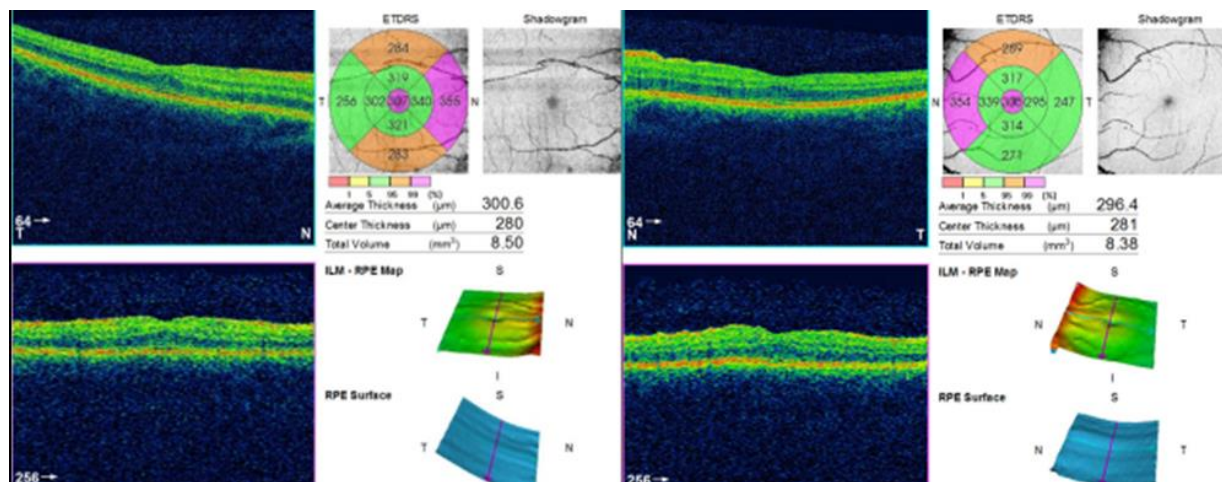
Figure 8.3 OCT appearance in ARSACS



OCT appearance in ARSACS showing both eyes of patient 1 (top) and patient 2 (bottom). This figure demonstrates: optic nerve photograph with peripapillary ring scan indicated with green circle, pie graph of quadrants with RNFL thickness and RNFL circular tomogram representing quantitative analysis of RNFL thickness. Peripapillary thickening of RNFL in both eyes of both patients is observed (black line). Normative data set: green area = 95% confidence interval, yellow area = 99% CI, red area = outside 99% CI. Quadrants: S = superior, N = nasal, I = inferior, T = temporal

In both individuals with ARSACS OCT confirmed that in the macula RNFL thickening extends over the fovea leading to obscuration of the foveal pit (Figure 8.4).

Figure 8.4 Macular appearance in ARSACS



Macular 3D scan in patient 1 demonstrating extension of the RNFL thickness over the fovea and obscuring the foveal pit bilaterally

Longitudinal assessment

Patient 1 had further OCT studies 28 months after initial assessment.

Table 8.3 shows the mean measurements from both eyes at the initial and follow-up assessments. There was a tendency for increased average macular and foveal thickness, while average RNFL thickness remained stable. No disability progression as quantified with SARA score was observed.

Table 8.3 Visual acuity, SARA score and OCT measurements in ARSACS patient at baseline and at follow-up

Pt 1	VA		SARA	Average macula thickness (μm)	Foveal thickness (μm)	Average RNFL thickness (μm)	Superior RNFL thickness (μm)	Nasal RNFL thickness (μm)	Inferior RNFL thickness (μm)	Temporal RNFL thickness (μm)
	R	L								
Baseline (27 yr)	20/30	20/40	12	298.6	306	132.5	129.5	141	145	114
Follow up (30 yr)	20/40	20/40	12	301.15	309	132	130	144	144	114.5

8.5 Discussion

This study described results of a comprehensive clinical, ophthalmological, radiological and genetic evaluation of patients with biallelic SACS variants attending the NAC, Ireland.

Two individuals with recessive pathogenic / likely pathogenic SACS variants had early onset spastic ataxia and axonal-demyelinating peripheral neuropathy, similarly to the described originally relatively homogeneous French-Canadian ARSACS cases [121]. Although a number of reports suggested that age at onset may be a little later in non-Québécois cases [262, 263, 264], the patients in this study had early childhood symptom onset.

According to the literature, ARSACS phenotype is more variable than originally thought. The classical triad includes very early-onset cerebellar ataxia, spasticity and axonal-demyelinating sensorimotor peripheral neuropathy, combined with increased visibility of retinal nerve fibre, while superior cerebellar atrophy and linear pontine hypointensities on T2 and T2-FLAIR weighed images are the characteristic radiological signs [265]. However, cases with adult onset, prominent cognitive impairment, hearing loss, and lack of ataxia, spasticity or peripheral neuropathy have been documented [262, 263, 265]. Furthermore, retinal changes are not consistently observed on funduscopy, particularly in non-Québécois cases of ARSACS. Thus, the phenotypic variability can make ARSACS difficult to diagnose.

In 2011, Pablo et al. described thickening of the RNFL as demonstrated with OCT [266] and suggested revision of the ARSACS diagnostic criteria to include retinal abnormalities. Increased RNFL thickness is a very unusual finding in neurodegenerative conditions. In a large British study [91] all ARSACS patients had abnormal RNFL thickening, implying that this is a hallmark of the disease. In the same year, Filho et al. published the results of a

Brazilian ARSACS cohort and also documented thickening of the RNFL in all of their patients, indicating that retinal involvement is not restricted to certain ethnic groups [128].

In this study, peripapillary striations were observed in the two individuals with typical ARSACS phenotype. These were more visible on the fundus images than on classic ophthalmoscopy. In contrast, the third individual, who had two VUSs in the *SACS* gene, presented considerably different phenotype consistent with an uncomplicated spastic paraplegia, and did not demonstrate RNFL thickening. Peripapillary RNFL thickening as detected with OCT was also not seen in any of the other types of inherited ataxias included in this project, suggesting that OCT has a very high specificity and sensitivity in ARSACS.

In the larger British study, OCT measurements of the average peripapillary RNFL thickness were significantly lower in the four cases where fundoscopy was clinically normal, similarly to patient 3 in this study. However, the underlying genetic diagnosis in this individual is still unknown and therefore, it is unclear if this is a case of ARSACS with normal appearing fundus and normal RNFL measurements, or a completely different entity. Moreover, the two individuals with classical ARSACS phenotype had the pathognomonic changes on the MRI, originally described in French-Canadians. In contrast, the third individual with two *SACS* VUSs, had different pattern on cerebellar involvement and normal appearing pons, thus lacking specific radiological abnormalities associated with ARSACS.

Visual function, disease severity and RNFL parameters did not change dramatically over time in patient 1, which could be explained by the slow rate of progression of ARSACS.

8.6 Conclusions

In summary, characteristic retinal findings seen on OCT can help guide genetic testing, as RNFL changes documented by the OCT appear to be a sensitive marker in distinguishing ARSACS from other genetic ataxias. Thus, routine use of OCT in the assessment of individuals with ataxia particularly in patients presenting with spastic ataxic phenotype should be considered.

Chapter 9: Clinical phenotype and optical coherence tomography in other ARCA and ARHSP

9.1 Introduction

9.1.1 ARCA

ARCA is a complex group of rare disorders with significant clinical and genetic heterogeneity. Notable advancements in the last decade in molecular genetics and in particular next- generation sequencing (NGS) techniques, have led to the identification of new ARCA causing genes and novel phenotypes of known ARCA-causing genes [267]. The latest classification acknowledges 59 primary ARCA disorders and FRDA is recognised as the most prevalent ARCA worldwide, followed by *SPG7*-associated spastic ataxia, Ataxia telangiectasia (A-T) and Ataxia with oculomotor apraxia (AOA) [5, 13, 17]. In the NAC, among non-FRDA ARCA, *SPG7*-associated spastic ataxia is the most common, followed by *ANO10*-associated spastic ataxia, A-T, AOA2, AOA1, ARSACS, and *DDHD2*-associated spastic ataxia [5].

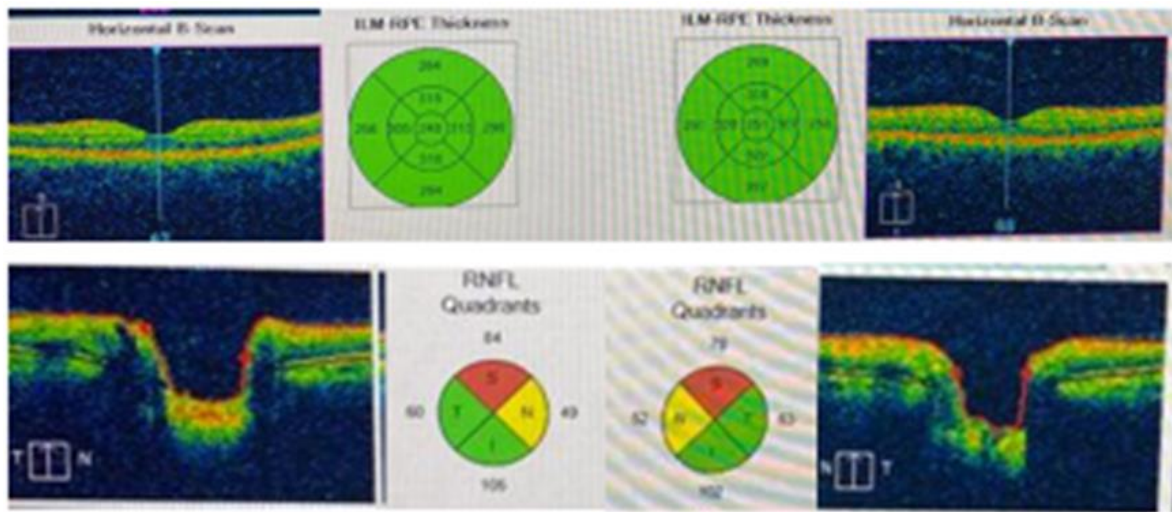
In the literature, data on retinal findings as detected by OCT in all non-FRDA, except for ARSACS, are sparse.

ANO10-associated spastic ataxia

Autosomal recessive cerebellar ataxia type 3 (ARCA3) is a rare inherited disorder caused by mutations in the *ANO10* gene. The disease is characterised by slowly progressive spastic ataxia variably associated with motor neuron involvement, epilepsy, and cognitive decline. A notable oculomotor finding in *ANO10*-associated ataxia is the presence of downbeat nystagmus, reported in more than 30 % of patients [268, 269]. More recently, tortuosity and telangiectasia of sclera and conjunctiva have been reported as part of the phenotype [270], indicating that these findings may be important discriminating factors to differentiate *ANO10*-associated ataxia from other spastic ataxia phenotypes without neuropathy. Retinal developmental abnormalities, felt to be unrelated to the ataxia phenotype, have been previously reported in one individual with bilateral macular hypoplasia on ophthalmological examination [271].

No prior reports describing RNFL findings in this entity have been published. However, unpublished OCT data from an Irish family with *ANO10* - associated phenotype [270], evaluated in The Royal Victoria Eye and Ear Hospital, Dublin, Ireland with Cirrus HD-OCT machine, demonstrated reduced global RNFL measuring 75µm on the right and 74µm on the left in one of the siblings (Figure 9.1). OCT data from other two siblings are limited due to poor image quality because of ocular motor abnormalities.

Figure 9.1 Macular and optic disc appearance in *ANO10*-associated spastic ataxia



Cirrus HD-OCT of individual with *ANO10*-associated spastic ataxia demonstrating normal macular appearance and measurements. 3D OCT shows reduced predominantly superior and nasal sectorial RNFL thickness

9.1.2 Other HSPs

HSP are clinically and genetically diverse group of disorders [6], characterised by a length-dependent distal axonopathy of the corticospinal motor neurons resulting in lower limb spasticity and weakness in pure forms. A large variety of additional neurological features, such as ataxia, optic atrophy, peripheral neuropathy or cognitive impairment, and non-neurological features may further complicate the phenotype, and these presentations are classified as complex or complicated HSP forms. In the last number of years the discovery of more and more genes causing both predominantly pyramidal and prominent cerebellar phenotypes has demonstrated the significant overlap between CA and HSP [129]. A number of genes, including *SPG7* and *SYNE1*, originally identified as a cause of HSP and CA

respectively, were subsequently found to cause ataxia on the one end of the disease spectrum and HSP on the other. Other genes, like *GBA2* and *KIF1C* were almost simultaneously reported as both HSP and ataxia genes. Therefore, cerebellar and pyramidal presentations commonly occur together and can vary considerably in predominance and phenotypic expression along the disease spectrum.

To date, a handful of OCT studies have investigated retinal changes in HSP patients to evaluate if RNFL thinning, seen in other neurodegenerative disorders, possesses any specificity [71, 77, 79].

Small case series have reported subclinical optic neuropathy even in HSP patients with little or no evidence of visual dysfunction, detected as an abnormal thinning of the RNFL to a degree measurable by OCT.

Wiethoff et al. [130] performed OCT in 28 HSP patients, clinically divided into pure (n=22) and complex form (n=6). Majority of the individuals had SPG4 (13), 1 patient had SPG5 and 3 patients had SPG7, while 11 individuals were genetically undetermined. Significant RNFL reduction was found in temporal and temporal inferior quadrants of patients with complex but not with pure HSP phenotypes, including two of the three individuals with SPG7. No correlation was found between global RNFL thickness and age of onset, disease duration and disease severity as quantified with the Spastic Paraplegia Rating Scale (SPRS). In a simultaneous multicenter study of 134 patients with HSP, 10 individuals with *SPG7*-associated phenotype underwent OCT and were found to have evidence of RNFL thinning [131]. No other individuals from this cohort were subjected to OCT.

More recently reported cohort of 23 genetically highly heterogeneous HSP patients included individuals with SPG4 (n = 8), SPG3a (n = 6), SPG72 (n = 3), SPG5 (n = 2), SPG7 (n = 2),

and SPG8 (n = 2) [132]. OCT analysis showed that thinning of the RNFL, which was mostly mild-to-moderate, was not a constant feature of complex HSP, but was more widespread and extended to the pure forms, and RNFL changes did not affect any specific quadrants. Observed RNFL thinning correlated with age and disease duration, but not with clinical severity as quantified with the SPRS.

ARHSP with thin corpus callosum (TCC) and intellectual disability is a phenotype commonly seen in SPG11 and SPG15. Recently, mutations in DDHD-domain-containing 2 (*DDHD2*, HGNC:29106) were linked to SPG54 (OMIM#615033), typically presenting with early-onset lower limb spasticity, intellectual disability, and TCC on brain imaging, while brain magnetic resonance spectroscopy demonstrates an abnormal lipid peak [272].

Although optic nerve hypoplasia was hypothesised as a possible frequent clue for SPG54 in the first descriptions [272], it has only been reported occasionally in the following cases [273]. No prior studies have reported OCT findings in *DDHD2* – associated spastic ataxia.

C12orf65 gene is involved in the process of mitochondrial translation. Mutations in this gene have been linked to a spectrum of phenotypes, including early onset optic atrophy, progressive encephalomyopathy, peripheral neuropathy, and spastic paraparesis, classified as the rare AR SPG55 [133]. OCT in affected individuals revealed general reduction in the RNFL thickness, mainly in the temporal area of the optic disc [134].

Kjellin syndrome is a neuroophthalmologic presentation linked to mutations in *SPG11* and *SPG15* genes, which was initially identified in association with pigmentary maculopathy and subsequently with an increased risk of developing psychosis [135]. OCT in patients with this syndrome showed that retinal changes are only observed once the paraplegia becomes apparent [136].

Optic atrophy has also been described in patients with SPG35 [137], but no studies have documented OCT findings.

9.2 Specific aims

The specific aims of this study were to describe patients with ARCA and HSP (excluding FRDA, ARSACS and *SPG7*-associated spastic ataxia, described in detail in Chapters 5, 6, and 8); to characterise their retinal findings using OCT; to determine whether the pattern of retinal changes (when present) in patients with other ARCA and ARHSP possesses any specificity.

9.3 Methods

All affected individuals had clinical assessment using a standardised approach, comprising demographic information, history, pedigree, and detailed neurological examination as outlined in Chapter 4.

The Scale for the Assessment and Rating of Ataxia (SARA, range from 0-40 points with higher scores indicating more severe disease) [99] (Appendix 3) was obtained for each affected individual.

The best-corrected visual acuity (BCVA) was measured using Snellen chart and results were expressed in logarithm of the minimal angle of resolution (LogMAR) for analysis.

Each participant had a spectral-domain OCT examination with Topcon 3D OCT-2000 as described in details in Chapter 4.

Descriptive statistics were used to summarise the characteristics of this group.

9.4 Results

The 8 patients with genetically confirmed ARCA/ARHSP included: ARCA3 (n = 1), SPG54 (n = 3), AOA1 (n = 2), and CANVAS (n = 2). Table 9.1 summarises visual acuity and OCT findings in evaluated patients. Good quality image scans were obtained from 6 individuals.

Table 9.1 Visual acuity and OCT findings in ARCA/ARHSP patients

Patient	VA		Average macula thickness (μm)	Foveal thickness (μm)	Average RNFL thickness (μm)	Superior RNFL thickness (μm)	Nasal RNFL thickness (μm)	Inferior RNFL thickness (μm)	Temporal RNFL thickness (μm)
	R	L							
ARCA3	20/25	20/20	260.95	258	87.5	97	85.5	110	57.5
SPG54	20/20	20/25	NA		64	92	59	71	34
SPG54	20/30	20/30	NA		76	75	86	70	74
AOA1	20/25	20/20	283.4	290	117	122.5	121	150.5	75
CANVAS	20/40	20/25	283.4	246	93	111	81.5	115	64.5
CANVAS	20/25	20/25	267.4	268	93	95	68	123	86
Controls	mean ± SD		270.92 ± 12.7	240.3 ± 26.7	102.9 ± 7.6	119.9 ± 11.3	90.1 ± 12	127.2 ± 11.7	74.8 ± 8.7
	(range)		(245 - 300)	(201.5 - 308.5)	(83 - 114)	(89 - 144)	(71 - 124)	(96 - 152)	(60 - 93)

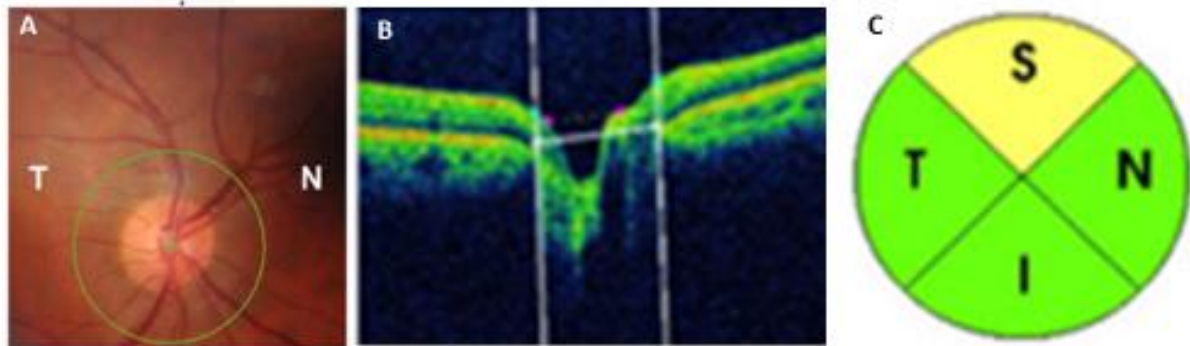
ANO10- associated spastic ataxia (ARCA3)

A 37-year-old man presented with progressive spastic ataxia with onset at 24 years of age. He had a single seizure at age of 33 years. On examination he had moderate cerebellar dysarthria, downbeat nystagmus and prominent tortuous ocular vessels with telangiectasia. Tendon reflexes were brisk, and plantar responses were flexor. He walked with a spastic ataxic gait. SARA score was 9.5. There was no clinical or neurophysiological evidence of neuropathy.

This patient had a homozygous pathogenic variant in the *ANO10* gene, c.132dupA p.(Asp45fs), which is the most common variant seen in the NAC at TUH [5].

OCT showed reduced global thickness of the RNFL (Table 9.1; Figure 9.2) and a tendency to predominantly superior quadrant thinning.

Figure 9.2 Optical coherence tomography of a patient with *ANO10*-associated spastic ataxia

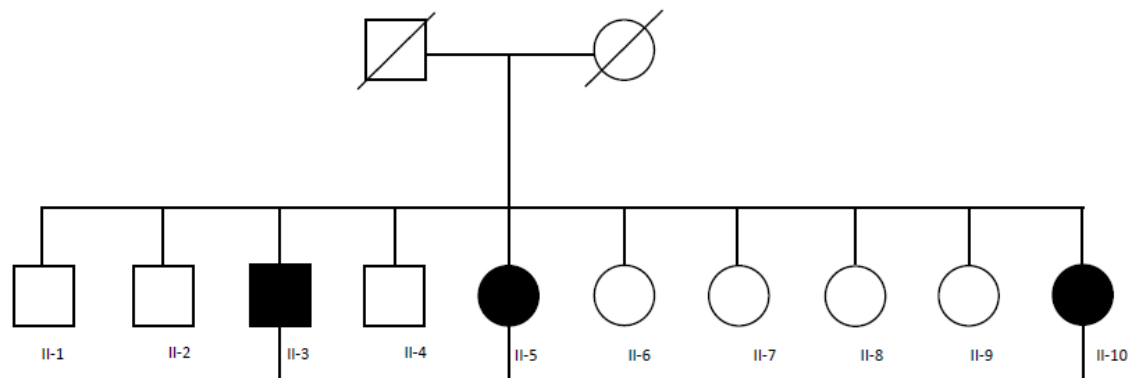


Optic nerve photograph (right eye) with peripapillary ring scan indicated with green circle (A); Horizontal tomogram (B); Pie graph of quadrants (C) demonstrating a tendency to predominantly superior RNFL reduction (normative data set: green area = 95% confidence interval, yellow area = 99% CI, red area = outside 99% CI). Quadrants: T = temporal; S = superior; N = nasal; I = inferior

DDHD2-associated spastic ataxia

One male (II-3) and two female (II-5 and II-10) siblings (Figure 9.3) from a non-consanguineous Irish family developed teenage-onset balance difficulty, followed by dysarthria and detrusor instability. Gait ataxia gradually progressed, requiring walking aid in their mid-to-late thirties. Academically, all attended a special school until 14 years of age, however, II-10 subsequently completed a secretarial course.

Figure 9.3 Pedigree of an Irish Family with *DDHD2*-associated phenotype



On examination (Table 9.2), affected siblings had oculomotor apraxia, lower limb spasticity, truncal and limb ataxia, variably present pes cavus, dystonia, distal wasting and weakness. All had brisk knee reflexes and extensor plantar responses. Pin and vibration were reduced distally in II-3, his sisters had no clinical evidence of neuropathy.

Targeted sequencing with a 91-gene panel (www.ouh.nhs.uk/geneticslab) revealed homozygous *DDHD2* variants, not recorded in Exome Variant Server, ExAC and dbSNP, in II-5. Segregation analysis confirmed these variants in II-3 and II-10. Parents were not available for testing. The c.1891+2T>C change disrupts canonical splicing donor site and is predicted to lead to aberrant splicing. An alternative homozygous *DDHD2* variant, also presumed to lead to aberrant splicing, has been reported in HSP with cerebellar signs [274].

Table 9.2 Phenotypic characteristics of 3 siblings with progressive cerebellar ataxia and spasticity due to homozygous *DDHD2* mutations

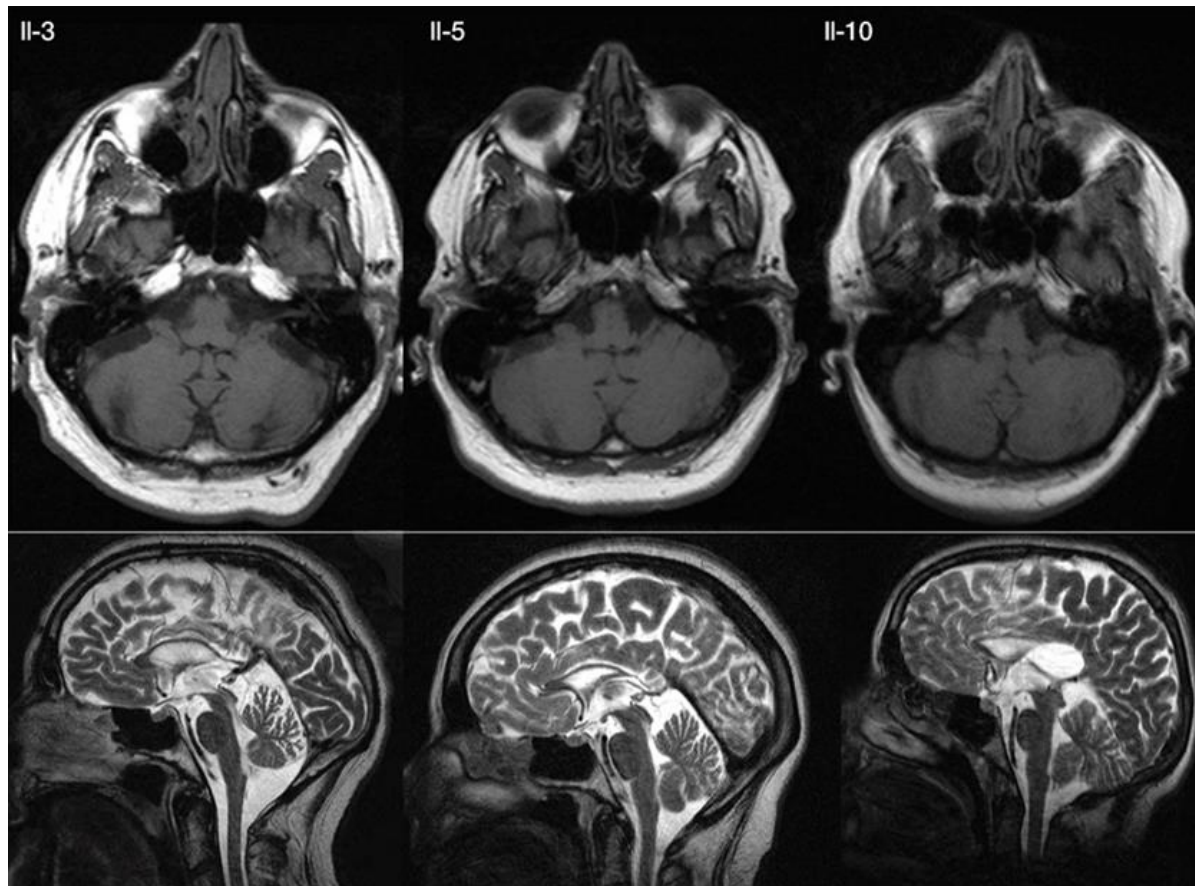
	II-3	II-5	II-10
Age at onset	14	12	14
Age at assessment	62	55	47
Ocular motor findings	Horizontal GEN SWJs Oculomotor apraxia Temporal pallor +	Horizontal GEN SWJs Oculomotor apraxia	SWJs Oculomotor apraxia Temporal pallor +
Red green colour deficiency	+	+	+
Cerebellar dysarthria	+++	++	++
Limb ataxia	+++	++	+
Reflexes UL	Increased	Increased	Increased
Reflexes LL	Increased knee jerks Absent ankle jerks	Increased knee jerks Absent ankle jerks	Increased
Plantar responses	↑↑	↑↑	↑↑
UL spasticity	+	+	–
LL spasticity	++	++	++
Autonomic dysfunction	Detrusor overactivity	Detrusor overactivity	Detrusor overactivity
Amyotrophy	Intrinsics, distal calves	–	–
Seizures	–	–	* +
Pes cavus	+	–	+
Dystonia	+	–	+
Dysphagia	+	+	–
Intellectual disability	+	+	+

NCS	Sensorimotor axonal length-dependent neuropathy	N	N
Brain MRI	TCC Mild cerebral and cerebellar atrophy	TCC Mild cerebral and cerebellar atrophy	TCC Mild cerebral and cerebellar atrophy

GEN, Gaze-evoked Nystagmus; SWJs, Square wave jerks; + Mild; ++ Moderate; +++ Marked; UL, Upper limbs; LL, Lower limbs; MoCA, Montreal Cognitive Assessment; N, normal; NP, not performed; TCC, Thin corpus callosum; *Symptomatic seizures attributed to frontal lobe cortical stroke

MRI brain showed TCC, mild generalised cerebral and cerebellar atrophy (Figure 9.4). Nerve conduction studies were normal in II-5 and II-10, and demonstrated sensorimotor large-fibre axonal neuropathy in II-3.

Figure 9.4 Magnetic resonance image of the brain in affected siblings with *DDHD2*-associated spastic ataxia



MRI brain of II-3, II-5, and II-10, performed at the age of 49, 43, and 34, respectively. Axial T1 views of the cerebellum are shown in the top row, and sagittal T2 views through the corpus callosum are shown in the bottom row. Thin corpus callosum is observed with additional mild generalised cerebral and cerebellar atrophy, most evident in the eldest and most severely affected sibling (II-3). Taken from [277]

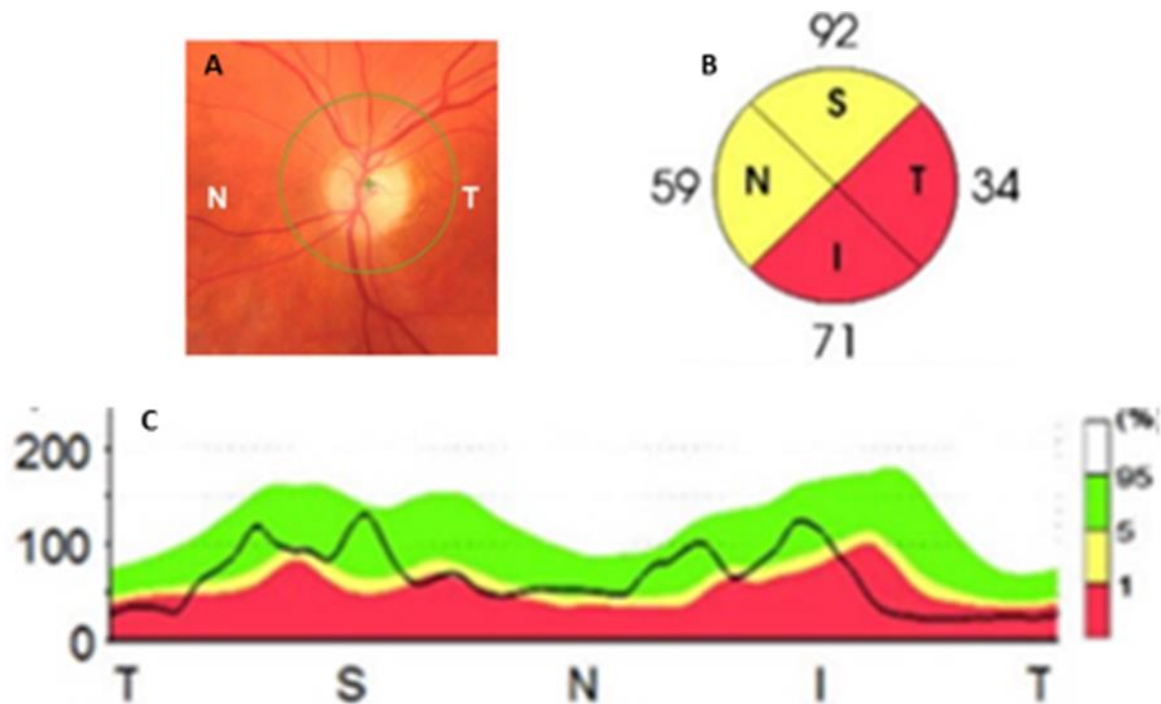
Cognitive assessment suggested significant multi-domain progressive cognitive decline. Reading ability on TOPF (premorbid intellect estimator) appeared an underestimate, based on their educational attainment. TOPF performances demonstrated impulsive responding, supported by executive dysfunction indications; no systematic errors were apparent

suggesting acquired reading difficulty; reading of individual letters was intact. The siblings' outcomes were compared with clinically unaffected age-matched controls of comparable educational attainment. Affected siblings showed most consistent marked impairment on executive function tasks. The youngest sibling exhibited least progressed cognitive impairment.

Ophthalmology assessment revealed normal visual acuity. Ishihara plate testing demonstrated red-green colour deficiency in all affected and was normal in their seven unaffected siblings.

OCT was attempted from both eyes in all three siblings. Due to the marked oculomotor apraxia and SWJ present in all, OCT studies were very challenging. Only two scans (3D optic disc scans from two siblings, one eye each) with good image quality (image quality score of $\geq 50/100$) (Table 9.1) were obtained. Analysis of these two scans showed reduced average peripapillary RNFL, as well as abnormal thinning in all sectors in one (II-10), and predominant superior and inferior quadrant involvement in the other individual (II-5) (Figure 9.5).

Figure 9.5 Optical coherence tomography of a patient with *DDHD2*-associated spastic ataxia



OCT appearance in II-10 (left eye): A. Optic nerve photograph with peripapillary ring scan indicated with green circle. B. Pie graph of quadrants with RNFL thickness. C. RNFL circular tomogram representing quantitative analysis of RNFL thickness (black line) and normative data set (green area = 95% confidence interval, yellow area = 99% CI, red area = outside 99% CI). Quadrants: T = temporal; S = superior; N = nasal; I = inferior

Ataxia with oculomotor apraxia type 1 (AOA1)

A male and female siblings from non-consanguineous Irish parents had childhood onset progressive balance difficulties (Table 9.3). They also displayed sensori-motor polyneuropathy, hypoalbuminaemia, elevated α -fetoprotein and hypercholesterolaemia. MRI brain showed cerebellar atrophy.

These two individuals had confirmed genetic diagnosis of AOA1 following Sanger sequencing of the *APTX* (Aprataxin) gene.

Table 9.3 Characteristics of individuals with AOA1

Patient	Pt 1	Pt 2
Sex	F	M
Age at onset	7	6
Age at exam	43	55
Disease duration	36	49
Age at wheelchair	18	29
Oculomotor apraxia	No	Yes
Hypometric saccades	No	Yes
Nystagmus	GEN (lateral)	GEN (lateral+vertical gaze)
LL weakness	Distal > proximal	Severe distal & proximal
Reflexes LL	Absent	Absent
Plantar responses	↓↓	↓↓
Neuropathy (NCS)	Yes	Yes
Other	Moderate LL oedema	Marked LL oedema
MRI cerebellar atrophy	Yes	Yes
Elevated AFP	Yes	Yes
Hypoalbuminaemia	Yes	Yes
Hypercholesterolemia	Yes	Yes
SARA	26.5	29

OCT was attempted on both eyes of each sibling. Due to marked oculomotor abnormalities images with good image quality were not obtained from Patient 2. The OCT results of

patient 1 are presented in Table 9.1. There was no abnormal thinning observed in the macular and optic disc areas.

Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS)

Two unrelated individuals from Irish origin presented with a late onset unsteady gait (Table 9.4), which was reported as being worse in the dark. Both patients developed chronic cough which preceded the onset of the walking difficulties.

On examination, both had signs in keeping with cerebellar dysfunction, large-fibre sensory neuropathy and vestibular impairment.

Extensive genetic testing revealed biallelic expansion of an intronic repeat in the *RFC1* gene, which is consistent with a diagnosis of CANVAS.

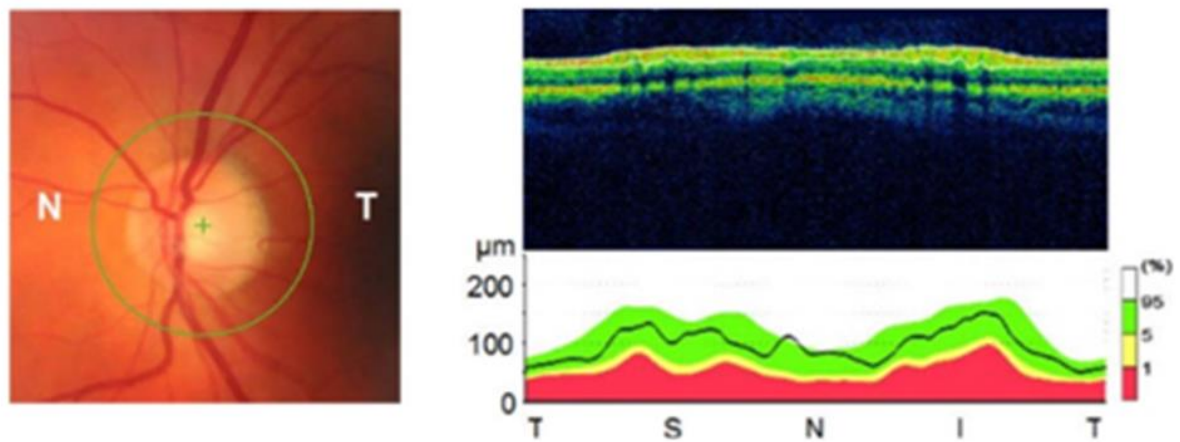
Table 9.4 Characteristics of individuals with biallelic *RFC1* repeat expansion

Patient	Pt 1	Pt 2
Sex	F	M
Age at onset	40 yr	52 yr
Age at exam	49 yr	57 yr
Onset of cough	25	38
Dysarthria	+	-
Oscilopsia	+	-
Vestibular impairment*	+	+
Sensory loss	Pin ↓ at knees Vibration ↓ at toe	Pin ↓ at knees Vibration ↓ at ASIS
Neuropathy (NCS)	Yes	Yes
MRI cerebellar atrophy	Yes	Yes
Other	Spasticity	Erectile dysfunction
SARA	7	3

*Abolished VOR (Vestibulo Ocular Reflex) at HIT (Head Impulse Test)

The results of OCT studies are presented in Table 9.1 and Figure 9.6. The average peripapillary, as well as sectorial RNFL thickness were within the normal range in these two individuals. Average macular and foveal thicknesses were normal.

Figure 9.6 Optical coherence tomography of a patient with CANVAS



OCT appearance in patient 2 (left eye), demonstrating optic nerve photograph with peripapillary ring scan indicated with a green circle, RNFL circular tomogram, and normal quantitative analysis of RNFL thickness (black line) with normative data set (green area = 95% confidence interval, yellow area = 99% CI, red area = outside 99% CI)

9.5 Discussion

This study investigated retinal involvement in patients with genetically confirmed ARCA/ARHSP using OCT. Most prominent and previously not reported RNFL changes were seen in the individuals with *DDHD2*-associated spastic ataxia, originally classified as SPG54. Previous OCT study of HSP cohort, including individuals with SPG4, SPG5, SPG7 and genetically undetermined cases, demonstrated that RNFL was affected in the majority of HSP patients with complex phenotypes where the disease spreads beyond the long fibre tracts [130]. In these cases significant thinning of RNFL and especially in the temporal sector was documented. This is also seen in one of the SPG54 patients studied here, who had the lowest temporal RNFL thickness among this heterogeneous group. Our family with homozygous *DDHD2* mutations developed a teenage-onset cerebellar ataxia as predominant feature in contrast to majority of SPG54 cases described thus far, who presented with spastic paraplegia and variable cerebellar signs, typically with very early onset [272, 274], although individuals from one family developed midlife-onset spastic ataxia without intellectual disability [275].

Cognitive decline, including executive dysfunction, although previously documented, has not been comprehensively characterised. This pattern of cognitive impairment is in keeping with reported frontal cortical *DDHD2* expression [272]. Furthermore, cerebellum's role in cognitive impairment is increasingly recognised [276] and in accordance with high cerebellar *DDHD2* expression.

To date, colour vision deficiency has not been previously reported in SPG54. In this family, colour blindness was shared only by affected siblings, suggesting that this may be a phenotypic clue in SPG54 patients [277].

A notable oculomotor finding in the *ANO10*-associated ataxia is the presence of downbeat nystagmus, which is reported in almost one third of patients [268, 269]. In contrast to previously described cases [268, 271], in which only tortuosity of ocular vessels has been reported, the individual with *ANO10* – associated spastic ataxia in this study had both tortuosity and telangiectasia of sclera and conjunctiva, similarly to other Irish patients sharing the same *ANO10* variant [270]. Telangiectatic vessels are distinct from tortuous vessels, in that the vessel wall is irregular in shape (i.e. parts of the vessel are dilated), whereas the tortuous vessels have a regular vessel wall but run an irregular course, and no part of the vessel is dilated. These findings may be important discriminating factors to differentiate ARCA3 from other spastic ataxia phenotypes without neuropathy. The OCT analysis showed reduced average RNFL thickness with a tendency to predominantly superior quadrant thinning, similarly to the findings in the FRDA cohort, where 44% of individuals had superior quadrant involvement. In contrast, patients with ARSACS, another spastic ataxia, exhibit abnormal RNFL thickening, while in *SPG7*- and *DDHD2*-associated spastic ataxias predominant temporal quadrant RNFL thinning has been observed. Altogether, these results imply that OCT might be a useful additional tool in distinguishing ARCA3 from other spastic ataxias.

AOA1 has been described as an early-onset ataxia with cerebellar atrophy, oculomotor apraxia (OMA), choreodystonia, and peripheral neuropathy. A recent genotype-phenotype study documented OMA in 61% of the included 80 patients and although not a universal finding, OMA was correlated with the SARA score, suggesting that OMA is a marker of an advanced AOA1 [278]. In this AOA1 family the female sibling did not display OMA, and notably, her SARA score suggested slightly less severe disease than in her brother, who

displayed OMA. Therefore, the absence of OMA should still be considered in patients with ARCA and neuropathy lacking OMA.

In addition to oculomotor abnormalities, macular and retinal changes, as seen on fundoscopy, have been documented in patients with AOA1. Optic atrophy has rarely been reported [279] and was not detected by OCT in the individual with AOA1 in this study.

Biallelic intronic AAGGG repeat expansions in replication factor complex subunit 1 (*RFC1*) have recently been described as a frequent cause of a late-onset ataxia [171]. An estimated allele frequency for CANVAS is in the range of FRDA, suggesting that pathogenic *RFC1* repeat expansions are a cause of a more common disease [280]. The phenotype is still evolving and an unexplained spasmodic dry cough was found to be present in over 60% of patients in a large study [280], suggesting that this should be considered as part of the associated phenotype. Cough was reported by both patients in this study, who otherwise presented with the full CANVAS phenotype, including cerebellar ataxia, peripheral neuropathy and vestibular failure.

There was no maculopathy or RNFL thinning observed, suggesting that afferent visual system is not involved in CANVAS.

9.6 Conclusions

In summary, the results from this study demonstrate that retinal thinning may occur in ARCA / ARHSP syndromes to a degree measurable by OCT. Furthermore, there is some evidence that different subtypes of ARCA / ARHSP exhibit distinct pattern of retinal thinning, thus supporting the importance of obtaining OCT especially in patients presenting with spastic ataxia.

Chapter 10: Clinical phenotype and optical coherence tomography in *AIFM1*-associated disease

10.1 Introduction

Mutations in Apoptosis-inducing factor, mitochondria-associated-1 (*AIFM1*) cause multiple distinct clinical phenotypes, including X-linked Charcot–Marie–Tooth disease type 4 (Cowchock syndrome, CMT4X) [281]. *AIFM1*-associated cerebellar ataxia has rarely been described [282, 283]. Individuals within families with *AIFM1*-associated disease typically develop symptoms in childhood or adolescence. In *AIFM1*-associated peripheral neuropathy neurophysiology usually demonstrates uniform axonal motor and sensory neuropathy, based on traditional evaluation of motor (tibial, peroneal, median and ulnar) and sensory (sural, median and ulnar) nerves [281, 284, 285], whereas radial sensory nerve was only reported as part of the evaluation in one individual [286].

Since gene discovery in 1999 [287], *AIFM1*-associated cerebellar ataxia has been reported in 24 individuals from 13 families and 83% of cases expressed spondyloepimetaphyseal dysplasia (SEMD) with central hypomyelination phenotype [282, 288].

No prior studies have reported OCT findings in *AIFM1*-associated disease.

10.2 Specific aims

The specific aims for this study [153] were to describe a large family with X-linked *AIFM1*-associated disease and to characterise their retinal findings using OCT.

10.3 Subjects and methods

Subjects

Seven affected and four unaffected family members were recruited for this study.

Clinical assessment

All affected individuals had clinical assessment using a standardised approach, comprising demographic information, history, pedigree, and detailed neurological examination as outlined in Chapter 4.

Charcot-Marie-Tooth (CMT) Neuropathy Score 2 (CMTNS2) [289] (Appendix 9), Scale for the Assessment and Rating of Ataxia (SARA) [99] (Appendix 3), colour vision testing with Ishihara test plate [173], Montreal Cognitive Assessment (MoCA) [290] (Appendix 10), and The International Standard Classification of Education (ISCED) were obtained from all affected.

Investigations

Nerve conduction studies (NCS) were acquired from all affected and two unaffected female carriers.

Genetic diagnosis

Molecular diagnosis was obtained through whole-genome sequencing (WGS) in one of the affected individuals (proband) and subsequent Sanger sequencing of the relevant variant performed in the other individuals.

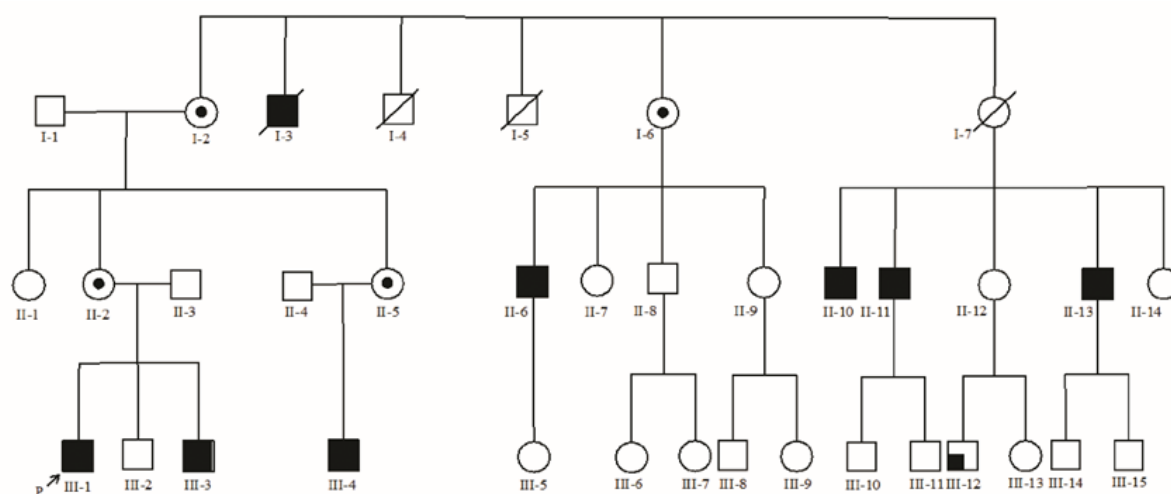
Optical coherence tomography

Spectral-domain optical coherence tomography (OCT) with Topcon 3D OCT-2000 was performed in all affected males and two of the asymptomatic females.

10.4 Results

Eleven related individuals (seven male) with a mean age of 51 years were identified and included in the study (Figure 10.1). There was no male-to-male inheritance.

Figure 10.1 Pedigree of the Irish Family with *AIFM1*-associated phenotype



Squares indicate male subjects; circles, female subjects; white circles and squares, healthy family members; black square, affected male subjects; white circle with black dot, asymptomatic female carriers; white square with black lower left symbol, deafness; symbol with a diagonal line, deceased individual; and the arrow, proband. Taken from [153]

A detailed summary of clinical characteristics of affected individuals are provided in Table 10.1. Male individuals presented with age of symptom onset ranging between 18 months – 39 years. All developed variably present progressive sensorineural deafness (onset between 4 - 34 years), length-dependent peripheral neuropathy with absent ankle reflexes, with or without distal wasting and glove and stocking sensory loss, cerebellar ataxia and pyramidal

involvement. Two had delayed walking and two individuals toe-walked. Poor balance was a presenting feature in one of the males. Clinical course was slowly progressive.

Mean age at assessment of affected males was 42 years. SARA ranged between 2-23/40, while CMTNS2 varied between 7-13/36. Cognitive assessment was normal in all. Neurological examination of obligate carrier females I-2, I-6, II-2 and II-5 was normal.

Nerve conduction studies

Neurophysiology (Table 10.1) showed symmetrical length-dependent large fibre sensory or sensory-motor peripheral neuropathy with most marked findings in the lower limbs. Distal motor responses in the lower limbs were small or absent in most of the affected individuals. Distal sensory responses were abnormal, more prominently in the lower limbs. NCS in the proband (III-1) also demonstrated bilateral carpal tunnel syndrome (CTS). The superficial radial responses were more markedly attenuated in all when compared with the median and ulnar sensory responses, even allowing for the CTS in III-1. There was no significant decline over a 12-year period in III-1 and III-4.

Neurophysiology of asymptomatic females II-2 and II-5 was normal.

Table 10.1 Phenotypic characteristics and neurophysiology findings of seven related individuals with cerebellar ataxia, deafness and neuropathy due to *AIFM1* mutation

Patient	III-1	III-3	III-4	II-6	II-10	II-11	II-13
Age at onset	18 months	16 yr	16 yr	17 yr	34 yr	25 yr	14 yr
Age at exam	36 yr	28 yr	39 yr	48 yr	49 yr	48 yr	44 yr
Age at walking	18 months	11 months	12 months (toe-walked)	12 months	12 months	12 months (toe-walked)	24 months
Presenting feature(s)	Hearing loss + poor balance	Hearing loss	Hearing loss	Hearing loss	Hearing loss (noticed after mild ear trauma)	Hearing loss (noticed after mild ear trauma)	Hearing loss
Onset of hearing loss	4 yr	16 yr	16 yr	17 yr	34 yr	25 yr	14 yr
Tinnitus	No	Yes	Yes	No	Yes	No	Yes
Onset walking difficulty	4 yr	21 yr	19 yr	22 yr	39 yr	always "funny" walk	always "funny" walk
EOM	Broken pursuits, slow saccades, SWJ	N	slow broken pursuits	N	N	N	N
Cerebellar dysarthria	++	+	—	—	—	—	—
Pes cavus	Yes	Yes	Yes	Yes	No	Yes	No
Hammer toes	Yes	Yes	Yes	Yes	No	Yes	No
Distal amyotrophy	Yes	Yes	Yes	Yes	No	No	No
Distal weakness	UL+LL	No	LL	UL+LL	No	EHL	EHL
Spasticity LL	Yes	No	Yes	Yes	No	Yes	No
Gait ataxia	+++	++	+	++	+	+	+
Limb ataxia UL / LL	+++	+ / ++	+ / +	+ / ++	+ / +	- / -	+ / +
Reflex loss	UL+LL	UL+LL	UL+LL	UL+LL	UL+LL	LL	UL+LL
Plantar responses	↑↑	Mute	↑↑	↑↑	↑↓	Mute	↑↑
Sensory loss	Vibration to cm JP to ankles	PP to ankles Vibration to cm	PP to mid shin Vibration to cm JP to ankles	PP to mid shin Vibration to cm	PP to distal 1/3 foot Vibration to cm	PP to mid foot Vibration to ASIS	PP to distal phal Vibration to cm
Ishihara test plate	Total colour blindness	N	Red-Green	Red-Green	N	N	N
MRI Brain (Age)	Cerebellar atrophy WM changes (30 years)	Subtle cerebellar atrophy (23 years)	Subtle cerebellar atrophy (33 years)	Subtle cerebellar atrophy (46 years)	NP	NP	NP
Additional features	Mild thoracic scoliosis		Mild thoracic scoliosis		-		
CMTNS2	9	10	13	13	10	7	11
SARA	23	8.5	6	11	6	2	5.5
MoCA	28	27	27	30	28	28	28
ISCED	ISCED 4	ISCED 3	ISCED 3	ISCED 5b	ISCED 3	ISCED 5a	ISCED 3

Neurophysiology findings																					
Motor Nerves	Lat (ms)	Amp (mV)	CV (m/s)	Lat (ms)	Amp (mV)	CV (m/s)	Lat (ms)	Amp (mV)	CV (m/s)	Lat (ms)	Amp (mV)	CV (m/s)	Lat (ms)	Amp (mV)	CV (m/s)	Lat (ms)	Amp (mV)	CV (m/s)	Lat (ms)	Amp (mV)	CV (m/s)
Right Median	3.55	7.2	58.5	3.08	8.7	57.4	2.85	10.8	55.8	3.81	4.9	54.1	2.44	8.3	58.1	2.66	8.9	55.3	3.19	4.6	51.2
Left Peroneal	5.07	0.18		7.66	2.4	40		—			—		3.64	0.87	48	3.5	2.8	45.2	4.88	1.65	41.5
Right Tibial	5.95	0.46		5.52	0.95	42.8		—		3.47	1.53	50.2	2.8	16.2	50.9	3.14	9.2	49.4	4.05	0.83	44.3
Left Deep Peroneal	3.38	6.2	47.1	3.8	6.6	57.3	3.16	4.2	58.7	3.33	5.4	61.4	2.1	6.6	56.1	2.99	4.6	61.3	2.82	5	41.8
Sensory Nerves	Lat (ms)	Amp (µV)	CV (m/s)	Lat (ms)	Amp (µV)	CV (m/s)	Lat (ms)	Amp (µV)	CV (m/s)	Lat (ms)	Amp (µV)	CV (m/s)	Lat (ms)	Amp (µV)	CV (m/s)	Lat (ms)	Amp (µV)	CV (m/s)	Lat (ms)	Amp (µV)	CV (m/s)
Left Median Digit III – wrist	3.45	5.2	37.1	2.33	7.8	55.8	2.66	4	54.9	2.39	3.8	53.6	2.01	10.1	66.2	2.21	6.5	65.2	2.79	5	57.9
Right Superficial Radial Forearm - snuffbox	1.59	6.1	59.1	1.63	8.9	55.2	1.82	4.8	61.5	1.41	4.2	56	1.88	7.8	43.1	1.22	11.6	60.7	2.67	6.2	57.4
Right Ulnar Digit V – wrist	1.97	4	57.9	1.74	2.7	64.4	2.05	2	56.6	2.3	1.19	47	1.78	4.6	66.3	2.09	3.6	59.3	2.63	2.4	56.6
Left Sural	0	1.41	32.6	2.98	1.74	36.6		—			—		2.46	1.63	45.9	2.32	2.7	49.1	3.38	0.62	45.5
SRAR	0.15			0.17			0			0			0.19			0.23			0.1		

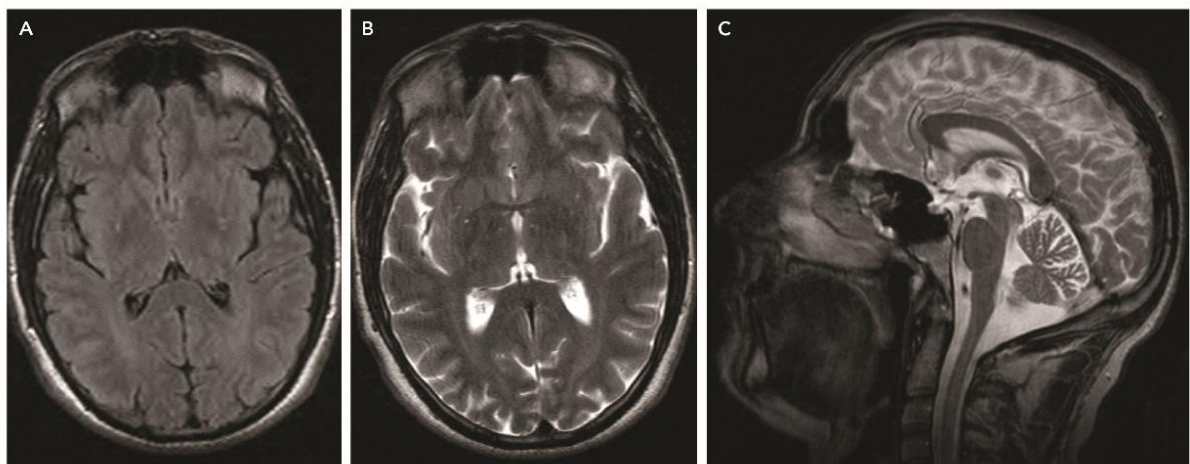
Abbreviations: EOM, Extraocular movements; SWJ, square wave jerks; UL, Upper limb; LL, Lower limb; N, normal; EHL, Extensor hallucis longus; +++, marked; ++, moderate; +, mild; PP, sensation to pin; JP, joint position sense; cm, costal margin; ASIS, anterior superior iliac spine; CMTNS2, Charcot-Marie-Tooth Neuropathy Score 2 (0 – 36); SARA, Scale for the Assessment and Rating of Ataxia (0 – 40); MoCA, Montreal Cognitive Assessment (0 – 30); ISCED, The International Standard Classification of Education; NP, Not performed; WM, white matter; Lat, latency; Amp, amplitude; CV, conduction velocity; m/s, metres per second; ms, milliseconds; mV, millivolt; μ V, microvolt; SRAR, Sural/Radial Amplitude Ratio
Abnormal values highlighted in **bold**.

Taken from [153]

Brain MRI

Brain MRI showed some white matter changes on FLAIR and T2 axial images and cerebellar atrophy in III-1 (Figure 10.2). Brainstem and corpus callosum were preserved. MRI brain of III-3, III-4 and II-6 showed subtle cerebellar atrophy.

Figure 10.2 Brain Magnetic Resonance Imaging of patient with *AIFM1*-associated phenotype



Patient III-1. A. Axial FLAIR image and B. Axial T2 weighted image demonstrating some white matter changes; C. Sagittal T2 weighted image demonstrating cerebellar atrophy.

Taken from [153]

Ophthalmological findings

Mean Snellen VA in 6 of 7 affected males was 20/25 (range 20/20 to 20/50), while in III-4 BCVA was 20/40 (right eye) and light perception (left eye). None of the affected individuals had disc pallor or retinitis pigmentosa on fundus examination. Two of the seven males included had abnormal ocular motor findings (III-1 and III-4, Table 10.1).

III-4 has a long standing complicated ophthalmology history, including surgery for blocked nasal lacrimal duct in infancy, treatment with oral steroids over the period of two years in

childhood, subsequent left eye secondary glaucoma associated with uveitis, and left eye trabeculectomy in adulthood.

Colour vision testing demonstrated red-green colour deficiency in III-4 and II-6, and total colour blindness in III-1; testing of other individuals was normal.

Optical coherence tomography

OCT of the affected individuals showed average and sectorial retinal nerve fibre layer (RNFL) thickness within the normal limits in all, but III-4, who had unrelated eye pathology.

Correlation with clinical features

There was no significant correlation between average macular and foveal, as well as average or RNFL thickness in different sectors and ataxia severity as quantified by SARA score, and neuropathy severity as quantified by CMTNS2. Similarly, no correlation was found between RNFL thickness and visual function (Table 10.2).

Table 10.2 Pierson's Correlation Coefficient between clinical features and OCT measurements

Clinical features	OCT parameters	<i>r</i> statistic	<i>p</i> value
CMTNS2	Average Macula	-0.42	0.35
	Fovea	-0.59	0.16
	Average RNFL	-0.58	0.17
SARA	Average Macula	0.69	.087
	Fovea	-0.12	.8
	Average RNFL	0.29	0.54
LogMAR	Average Macula	0.74	.06
	Fovea	-0.13	.78
	Average RNFL	0.32	.48

Genetic results

WGS of III-1 revealed a previously reported hemizygous variant in the *AIFM1* gene, p.Met340Thr. This is a missense variant, localised within a highly conserved amino-acid cluster. It is not seen at any allele frequencies in any of the large genome databases. Analysis for mutations in other genes associated with colour vision deficiency was negative. Subsequent Sanger sequencing confirmed this variant in the other six affected males. I-2, I-6, II-2 and II-5 were confirmed heterozygous carriers.

All affected individuals were commenced on supplementation with riboflavin 200 mg/day based on a small number of reports [283]. After two years there was no subjective or objective clinical improvement in our patients.

10.5 Discussion

To our knowledge, this is the first study to assess the role of OCT as a tool for detecting RNFL thickness changes in *AIFM1*-associated disease. The results show that RNFL thickness tends to be normal in *AIFM1* – associated presentations and there is no correlation between OCT parameters and disease severity as quantified with scales addressing the cardinal clinical features of this highly clinically variable complex syndrome.

This large family with *AIFM1*-associated disease demonstrated significant intra-familial phenotypic variability including novel associated features.

Consistent unique neurophysiological pattern of nerve involvement was observed and the superficial radial responses were preferentially attenuated even in very mildly clinically affected individuals, suggesting that this may be a characteristic feature of CMTX4. Predominant radial nerve involvement is unusual in genetic neuropathies which are typically length-dependent [251]. In contrast to our family, Wang et al reported two relatives with isolated axonal sensorimotor neuropathy and predominant involvement of the peroneal nerve [285]. Only one study described radial sensory nerve testing in an individual with *AIFM1*-associated motor neuropathy. Although sensory responses were reported as normal, the radial response at 12.5 μ V was reduced in comparison with the median (23.5 μ V) and sural (10 μ V) responses [286].

Hearing loss has been described in other X-linked CMT. In CMTX5, part of the *PRPS1*-related disease spectrum, patients develop early bilateral profound sensorineural deafness and peripheral neuropathy typically with onset before twelve years [291] in addition to optic atrophy, which was not observed in our family. Hearing loss is rarer in CMTX1, the most common X-linked dominant form of CMT, and in contrast to women who may have a milder phenotype [292], the radial sensory response in older males appears to be more severely affected compared with younger males [293] unlike in our family.

Families with *AIFM1*-associated SEMD with neurodegeneration exhibit notably homogeneous clinical features, including distinct dysmorphic appearance, skeletal abnormalities, and hypomyelination [282, 288]. In contrast, III-1 and III-4 had only very mild thoracic scoliosis. In our family only one individual had white matter abnormalities on MRI which were relatively non-specific and not felt to be typical for the appearances of hypomyelination.

The c.1019T>C p.(Met340Thr) mutation has been previously reported as causing deafness, progressive ataxia, and axonal neuropathy [283] with no features such as colour blindness or the preferential radial nerve involvement observed in our family. This hemizygous variant causes an amino-acid change from Met to Thr at position 340. In an alternative transcript this variant is located at position c.2T>C and causes loss of the start codon. In our family maternal inheritance of the mutation was confirmed.

The colour vision deficiency in some of the affected individuals suggests either a second X-linked condition, or a novel feature of the *AIFM1* phenotype. Analysis of other genes associated with colour vision deficiency did not detect any other clinically relevant variants suggesting that colour blindness may be due to the *AIFM1* mutation.

AIFM1 is a mitochondrial intermembrane protein involved in oxidative phosphorylation [287]. Colour vision deficiency, documented in three of the affected family members, is also seen in other mitochondrial disorders including Leber's hereditary optic neuropathy (LHON) and dominant optic atrophy (DOA), usually caused by *OPA1* mutations [214]. This mitochondrial link supports the role of this *AIFM1* mutation in colour vision loss.

In the three affected individuals who displayed colour blindness, dyschromatopsia was evident from a very early age, therefore correlation between colour deficiency and disease duration cannot be established. It is unclear how a single mutation can cause the observed intrafamilial heterogeneity, but significant phenotypic variation is seen in other conditions

[214, 292]. It is possible that other genes or environmental factors modify the *AIFM1* phenotypic expression.

In addition to reported cases of X-linked CMTX5 patients, optic atrophy has been described in association with impairment of colour vision in individuals with CMT2A, the most common type of AD axonal CMT due to mutations in *Mitofusin 2 (MFN2)*, a protein on the outer mitochondrial membrane [239].

In contrast to other complex ataxia syndromes, no visual pathway involvement was seen and no structural retinal changes were found on OCT except in an individual with unrelated eye pathology. Therefore, our OCT data suggest that retinal thinning is not a feature of *AIFM1*–associated disease.

10.6 Conclusions

This report highlights that cerebellar ataxia may occur as a leading clinical feature in *AIFM1*–associated disease and that significant intra-familial variability can exist. This report further expands the *AIFM1*-associated phenotype adding colour vision deficiency as a significant feature. The results from this study suggest that retinal thinning is not a disease-specific finding. However, consistent preferential involvement of the radial sensory nerve, despite significant clinical heterogeneity, appears to be a valuable clue to the *AIFM1*-associated neuropathy and should be included as part of the standard neurophysiology evaluation in suspected X-linked CMT.

Chapter 11: Clinical phenotype and optical coherence tomography in mitochondrial disorders and genetically undetermined ataxias

11.1 Introduction

11.1 Mitochondria and mitochondrial dysfunction in inherited neurodegenerative disorders

There is an increasing number of publications in the literature showing that mitochondrial dysfunction has an important role in a significant proportion of inherited neurodegenerative disorders.

Mitochondria are ubiquitous tubular-shaped double membrane intracellular organelles with a fundamental function in cellular respiration and energy production [294]. Their role in providing most of the adenosine triphosphate (ATP) requirements can explain why tissues, highly dependent on aerobic production, such as ocular structures, are preferentially affected [295]. Optic nerve involvement is a common feature of mitochondrial disease and, in a substantial portion of cases, is associated with profound visual loss [296]. Optic neuropathies in inherited neurodegenerative disorders associated with mitochondrial dysfunction often demonstrate preferential loss of the nerve fibres that serve central vision, colour vision, and contrast sensitivity for high spatial frequency [96], resulting in reduced vision. These are the axons of the P-ganglion cells which predominate in the macular region [297], as already discussed in Chapter 6. However, in other neurodegenerative disorders with mitochondrial dysfunction, including FRDA, these fibres are not affected [96].

Mitochondrial disorders can result from mutations in both the mitochondrial (mtDNA) and the nuclear DNA (nDNA), since both genomes code for mitochondrial proteins. The high-

copy number mitochondria genome is unique, with hundreds to thousands of mtDNA molecules per cell, depending on their specific energy requirements, and mtDNA replicates continuously and independently of nuclear genome replication [298].

It is estimated that mutations in the mtDNA are responsible for 15-30% of childhood onset and over 50% of adult-onset cases with mitochondrial disorders [299, 300].

11.1.2 Syndromic and non-syndromic mitochondrial optic neuropathies

Leber's hereditary optic neuropathy (LHON) and dominant optic atrophy (DOA) are the two most common mitochondrial hereditary optic neuropathies with mono symptomatic expression [211]. Prior studies have demonstrated the usefulness of OCT as an objective measure in the natural history of LHON [301]. Milea et al. have also shown that quantification of the RNFL thickness can be particularly helpful in DOA cases with equivocal morphological disc features [302].

As part of a more complex syndrome, optic neuropathy is common in multi-systemic mitochondrial disorders, including LHON / dystonia / Mitochondrial Encephalomyopathy Lactic Acidosis Stroke-like (MELAS)/Leigh overlapping syndrome [217]. Optic neuropathy has also been documented in mitochondrial diseases due to mutations in nuclear genes encoding mitochondrial proteins such FRDA (discussed in Chapter 5), deafness–dystonia–optic atrophy (Mohr–Tranebjerg) syndrome (MTS), *SPG7*-associated spastic ataxia (discussed in Chapter 6), DOA “plus syndromes”, CMT2A with optic atrophy or hereditary motor and sensory neuropathy type VI (HMSN VI) [214, 217].

11.2 Specific aims

The specific aims of this study were to describe patients with mitochondrial disease and individuals with suspected but genetically undetermined ataxic syndromes; to characterise their retinal findings using OCT; to determine whether the pattern of retinal changes (when present) in patients with mitochondrial disorders possesses any specificity; to determine if retinal changes detected by OCT are evident over time.

11.3 Methods

All affected individuals had clinical assessment using a standardised approach, comprising demographic information, history, pedigree, and detailed neurological examination as outlined in Chapter 4.

The Scale for the Assessment and Rating of Ataxia (SARA, range from 0-40 points with higher scores indicating more severe disease) [99] (Appendix 3) was obtained for each affected individual.

The best-corrected visual acuity (BCVA) was measured using Snellen chart and results were expressed in logarithm of the minimal angle of resolution (LogMAR) for analysis.

Each participant had a spectral-domain OCT examination with Topcon 3D OCT-2000 as described in details in Chapter 4.

Follow up assessment was performed in 1 individual.

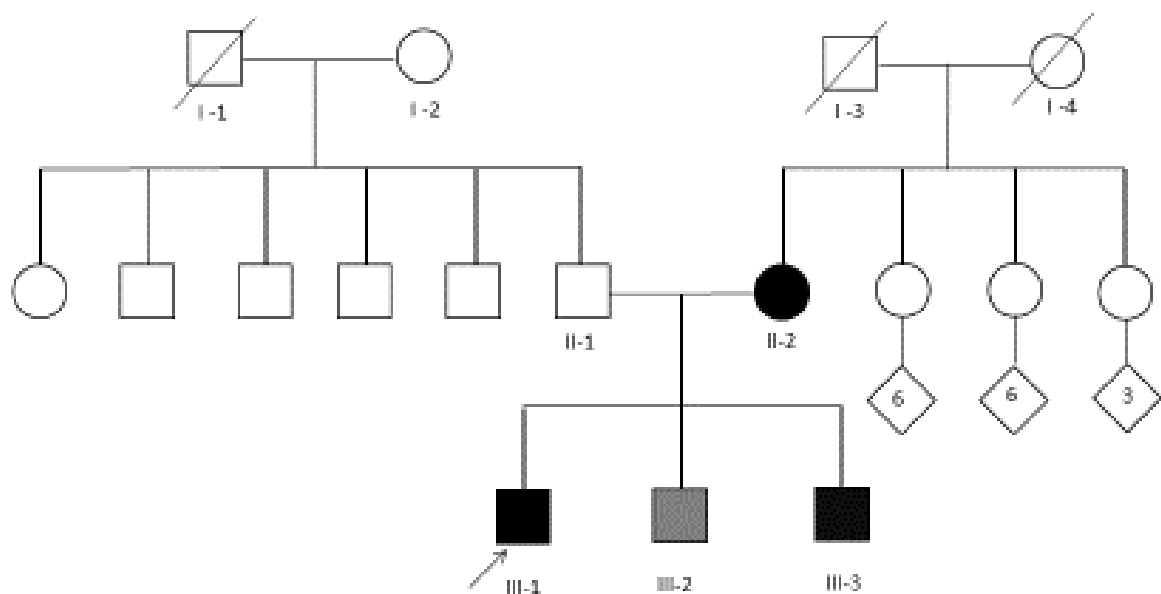
Descriptive statistics were used to summarise the characteristics of this group.

11.4 Results

11.3.1 m.8851T>C associated phenotype

Four related individuals (three affected and one clinically unaffected) from a single Irish family were identified and included in the study (Figure 11.1).

Figure 11.1 Pedigree of an Irish family with m.8851T>C mutation



Squares indicate male subjects; circles, female subjects; white circles and squares, healthy family members; black squares, affected male subjects; black circle, affected female subject; grey square, asymptomatic mutation carrier

Of the four individuals, three had variable age of symptom onset, between 3 and 25 years, and all presented with balance difficulty (Table 11.1). All four individuals had foot deformity. Cerebellar ataxia, length-dependent axonal peripheral neuropathy, ptosis, spasticity, scoliosis, dystonia, dysphagia, tortuous conjunctival vessels and primary gonadal failure were variably present. SARA score ranged between 1-10/40.

No retinopathy or disc pallor were seen in the individuals from this family (Figure 11.2).

Table 11.1 Characteristics of individuals with m.8851T>C mutation

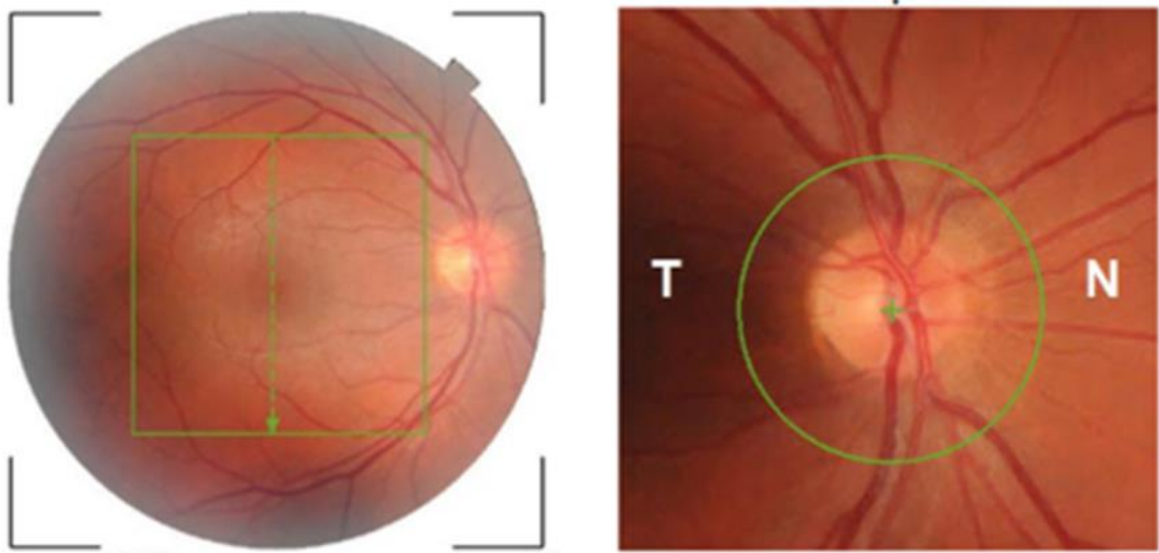
Patient	III-1	III-2	III-3	II-2
Age at onset	25 yr	No symptoms	3 yr	15 yr
Age at exam	32 yr	30	19	56
Ptosis	Bilateral L>R	-	-	Bilateral L>R
Dysarthria	+	-	-	++
Gait ataxia	+	-	-	++
Limb ataxia UL / LL	+ / +	- / -	+/-	+
LL weakness	EHL 4/5	-	-	Mild proximal & distal weakness
Spasticity LL	+	-	-	-
Reflexes LL	KJ ++ / AJ-	KJ ++, AJ ++	KJ +++ / AJ +	KJ - / AJ -
Plantar responses	↓↓	↓↓	↓↓	↓↓
Foot deformity	pes cavus / hammertoes	hammertoes	pes cavus / hammertoes	-
Sensory loss	Pin to ankles Vibration to knees	-	-	Pin to mid palms and ankles Vibration to ASIS JP to ankles
Neuropathy (NCS)	Yes	N/P	-	Yes
Other	tortuous conj vessels; scoliosis; gonadal failure	-	-	dysphagia, dystonic posturing
SARA	9	1	2.5	11.5

KJ, knee jerks; AJ, ankle jerks; NCS, Nerve conduction studies; SARA, Scale for the Assessment and Rating of Ataxia; -, Absent; + Mild, ++ Moderate, +++ Marked; N/P, not performed

Genetic analysis

WES with mitochondrial genome sequencing in III-1 detected a previously reported m.8851T>C p.(Trp109Arg) *MT-ATP6* pathogenic variant in a homoplasmic state. Further tissue sample testing from all family members confirmed very high level of heteroplasmy / possible homoplasmy in urinary epithelium, buccal epithelium and blood in all; in addition, a very high level of heteroplasmy / possibly homoplasmy was seen in the skeletal muscle in the mother (II-2), confirming the diagnosis of m.8851T>C-related disease.

Figure 11.2 Fundus image and optic disc appearance in a patient with m.8851T>C mutation



Nerve conduction studies

Neurophysiology, performed in three of the individuals demonstrated length-dependent large-fibre axonal sensory (in III-1) and sensorimotor (in II-2) neuropathy, while NCS in III-3 were normal.

Optical coherence tomography

The results of OCT studies are shown in Table 11.2. The average peripapillary, as well as sectorial RNFL thickness was within the normal range in the three male individuals; acceptable quality RNFL images from clinically most severely affected mother were not obtained. Average macular and foveal thickness were normal in all four individuals.

Table 11.2 Ophthalmological findings and RNFL thickness measured by OCT in patients with m.8851T>C mutation

Patient	III-1	III-2	III-3	II-2
Visual acuity R	20/20	20/20	20/40	20/25
Visual acuity L	20/20	20/25	20/20	20/50
Fundoscopy	N	N	N	N
Average macula thickness (μm)				
OD	281.6	282.2	294	262.1
OS	282.7	282.9	293.3	NA
Foveal thickness (μm)				
OD	248	242	255	223
OS	245	244	283	NA
Average RNFL thickness (μm)				NA
OD	100	102	94	
OS	102	93	92	
Superior RNFL thickness (μm)				NA
OD	102	112	102	
OS	122	104	118	
Nasal RNFL thickness (μm)				NA
OD	78	94	88	
OS	70	90	80	
Inferior RNFL thickness (μm)				NA
OD	132	136	119	
OS	124	116	109	
Temporal RNFL thickness (μm)				NA
OD	86	68	67	
OS	92	62	59	

Visual acuity (Snellen), presented as best-corrected visual acuity; OD, right eye; OS, left eye; N, normal; NA, not available

Longitudinal assessment in patient III-1

Patient 1 had further OCT studies 22 months after initial assessment, performed at 29 years. Table 11.3 shows the mean OCT measurements from both eyes at baseline and at follow-up. His visual acuity remained unchanged over time. The average and RNFL thickness measurements in different sectors remained similar, and no tendency to progressive thinning was observed both in the macular and the optic disc regions. His SARA score showed some

disability progression over the period, from 6/40 to 9/40 with advancements in the speech, stance and upper limb ataxia sub scores.

Table 11.3 Visual acuity, SARA score and OCT measurements in patient III-1 at baseline and at follow-up

III-1		VA		SARA	Average macula thickness (μm)	Foveal thickness (μm)	Average RNFL thickness (μm)	Superior RNFL thickness (μm)	Nasal RNFL thickness (μm)	Inferior RNFL thickness (μm)	Temporal RNFL thickness (μm)
		R	L								
Baseline	(29 yr)	20/20	20/20	6	282.15	246.5	101	112	74	128	89
Follow up	(31 yr)	20/20	20/20	10	285.75	250	101.5	115	74.5	128	89

11.3.2 Genetically undetermined ataxias

Sixteen patients with genetically undetermined ataxia were included. There was a female predominance in this group, 12/16 (Table 11.4). Equal number of patients had early (<20 years) and late symptom onset. The majority of patients (15/16) had disease duration, defined as the number of years since the first onset of gait instability of 10 or more years. Disease severity as quantified with SARA score varied between 2 and 27/40. BCVA (expressed in LogMAR) was 0.17 ± 0.21 (range 0 to 0.7) vs 0.05 ± 0.07 (-0.1 to + 0.3) in controls ($p=0.07$).

Optical coherence tomography

Results of the OCT studies are presented in Table 11.4. 3D optic disc scans from 10 eyes / 6 patients were excluded from analysis due to poor image quality (image quality score of $\leq 50/100$).

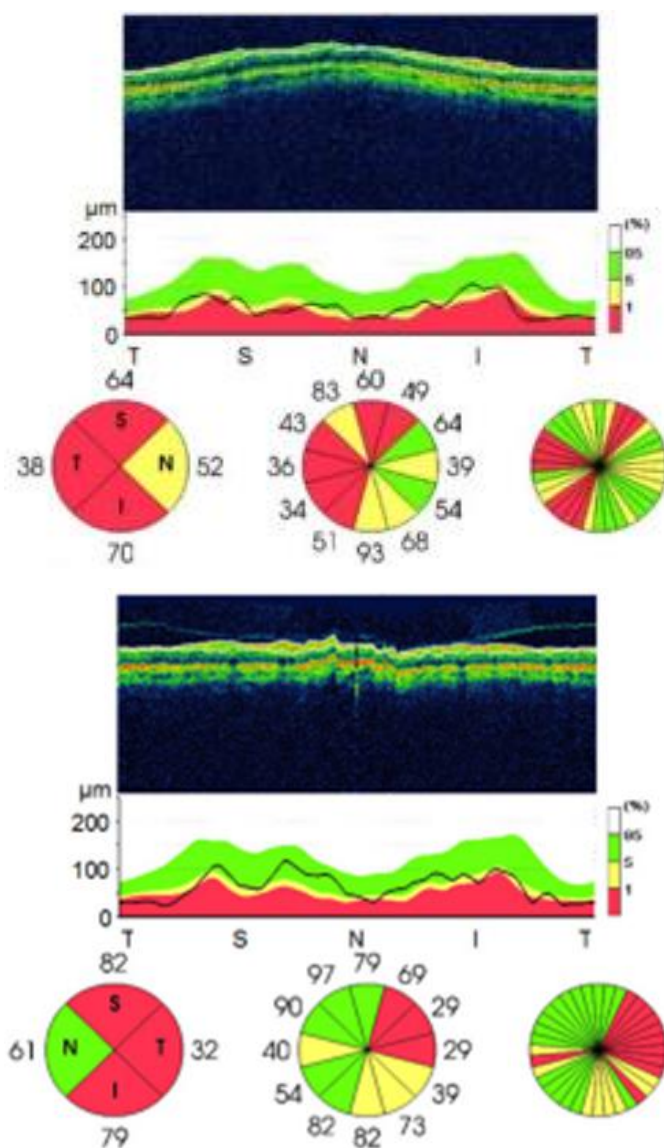
Table 11.4 Demographic, disease and OCT characteristics of individuals with undiagnosed ataxia

Patient	Sex	Age at onset	Age at assessment	Disease duration	SARA	VA R L	Average macula thickness (μm)	Foveal thickness (μm)	Average RNFL thickness (μm)	Superior RNFL thickness (μm)	Nasal RNFL thickness (μm)	Inferior RNFL thickness (μm)	Temporal RNFL thickness (μm)
1	M	9	19	10	10	20/20 20/20	280.7	251	107.5	131	79	135.5	82.5
2	M	12	44	32	16	20/25 20/20	294.8	256.5	106.5	128	111	135	68.5
3	F	3	40	37	27	20/25 20/30	267.8	257	94	126	66	116	68
4	F	14	45	31	17	20/70 20/40	269.3	268.5	NA				
5	F	50	62	12	13.5	20/20 20/20	278.55	296	92	87.5	75	128	78
6	F	35	62	27	9.5	20/25 20/25	269.75	226.5	99	111.5	88.5	115	81
7	M	58	61	3	13.5	20/20 20/30	279.85	230	93.5	113	92	103.5	68
8	F	45	59	15	10.5	20/40 20/25	268.15	215	94.5	102.5	97	113.5	63.5
9	F	1	49	48	15	20/40 20/30	252.15	240.5	NA				
10	F	1	44	43	10	20/20 20/25	262.4	263.5	NA				
11	M	2	22	20	9	20/20 20/20	283.2	232.5	97	101	97.5	112	74
12	F	10	28	18	3.5	20/70 20/50	289.05	283.5	NA				
13	F	41	54	13	7.5	20/20 20/25	273.4	245	94	106	92	108	69
14	F	40	50	10	27	20/100 20/70	241.4	238	70	106	65	64	46
15	F	22	39	17	2	20/20 20/20	232.8	189	85	107.5	73	84	75
16	F	57	70	13	2.5	20/30 20/30	225	219	60	73	56.5	75.5	35
Mean ± SD		25 ± 21	46.8 ± 14.8	21.8 ± 13	12.1 ± 7.4	20/30	266.8 ± 19.8	244.5 ± 26.8	91.1 ± 13.8	107.8 ± 16.6	82.7 ± 16.2	107.5 ± 22.6	67.4 ± 14
Controls							270.92 ± 12.7 (245 - 300)	240.3 ± 26.7 (201.5 - 308.5)	102.9 ± 7.6 (83 - 114)	119.9 ± 11.3 (89 - 144)	90.1 ± 12 (71 - 124)	127.2 ± 11.7 (96 - 152)	74.8 ± 8.7 (60 - 93)

Age at onset, age at exam and disease duration are presented in years

The average peripapillary RNFL thickness was reduced in patients, compared to controls ($p=0.01$). Abnormal thinning was also seen in all RNFL sectors. The average macular thickness was reduced in individuals with progressive ataxia in comparison to controls.

Figure 11.3 Optical coherence tomography of a patient with adult onset familial spastic ataxia



Optic disc scan in patient 16 (right eye - top, left eye - bottom) showing RNFL circular tomogram, quantitative analysis of RNFL thickness (black line) and normative data set (green area = 95% confidence interval, yellow area = 99% CI, red area = outside 99% CI),

demonstrating RNFL thickness below the normal limits in all except the nasal quadrant on the left.

11.5 Discussion

Mitochondrial DNA point mutations are a significant contributor to human disease with some population studies demonstrating a prevalence of greater than one in 200 live births [303]. Mitochondrial transfer RNA (mt-tRNA) genes constitute around 9% of the entire mitochondrial genome, have a crucial role in mitochondrial protein synthesis, and when mutated, can produce a wide diversity of clinical phenotypes [304] with onset in childhood or adulthood [305]. In most cases, multiple highly oxidative tissues are affected resulting in multisystem disease [306].

The *MT-ATP6* gene of the mitochondrial DNA (mtDNA) encodes a subunit of the F_1F_0 ATP synthase complex, a key enzyme of mitochondrial energy metabolism. Since the first description of a pathogenic m.8993 T > G variant in 1992 [307] more than 500 cases of *ATP6*-associated disease have been reported. The phenotypic spectrum is broad, including Leigh syndrome, NARP (Neuropathy, ataxia, and retinitis pigmentosa), Charcot-Marie-Tooth disease [308], Spinocerebellar ataxia with upper motor neuron signs [309], as well as combinations of ataxia, neuropathy, diabetes mellitus, and hypergonadotropic hypogonadism; and mitochondrial myopathy, lactic acidosis, and sideroblastic anemia [310].

Maternally inherited missense m.8851T>C variant has been reported only twice in the literature before. In contrast to this family, failure to thrive, psychomotor retardation and early onset choreoathetosis with bilateral striatal lesions were described in a 2.5 year old boy [311], while a 3 year old girl who presented with ataxia and Leigh syndrome [312], subsequently developed life-threatening hyperammonemia, which is uncommon in patients with mitochondrial disorders [313].

Although the middle brother in this family has very high level of heteroplasmy / possibly homoplasmy, he has very little signs. Asymptomatic carriers of homoplasmic pathogenic

variants have been reported in larger cohorts of individuals with *MT-ATP6* – associated disease [310], suggesting that the degree of mutation heteroplasmy does not strictly correlate with the severity of the disease, which may be influenced by environmental and other factors.

The OCT results in this family suggest that optic neuropathy is not a feature of m.8851T>C – related mitochondrial disease. However, the absence of retinal involvement here might be due to phenotypic variability commonly seen in mitochondrial disorders.

The group of patients with progressive ataxia who remain genetically undiagnosed is clinically highly heterogeneous and correspondingly, OCT findings ranged from normal to notably abnormal with RNFL thinning. Thus, it is essential to continue pursuing genetic diagnosis in these cases to allow interpretation of the retinal findings in the context of established genetic diagnosis.

11.6 Conclusions

The results of this study suggest that although optic nerve involvement is a common feature of mitochondrial disease and is often associated with significant visual loss, m.8851T>C mutations in the *MT-ATP6* are not associated with decreased visual acuity or RNFL abnormalities. However, considering the small sample size, low prevalence of the disease and its heterogeneity, these results should be interpreted with caution.

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Chapter 12: Conclusions and suggestions for future work

The main hypothesis underlying this thesis was that retinal changes in various types of inherited ataxias are more common than previously thought; that OCT may play a role in distinguishing different types of ataxias, and that RNFL structural changes have a functional correlate, and thus retinal thickness as measured by OCT has the potential to become a useful biomarker in CA.

In order to test this hypothesis, I documented the clinical phenotype of rare inherited ataxias by assessing 131 individuals with different types of genetically determined or suspected genetic ataxia, and 7 asymptomatic first-degree relatives of affected patients; I documented the retinal findings in affected individuals as detected by OCT and determined whether the peripapillary RNFL and macular thickness possessed any specificity, and whether OCT measurements were useful as imaging biomarkers in the assessment of patients with different types of CA. In addition, I documented disease duration and functional disability as quantified with SARA score, and their associations with OCT parameters as an anatomical marker in individuals with different types of inherited ataxia.

12.1 Optical coherence tomography in Friedreich's ataxia

In Chapter 5, I demonstrated that optic neuropathy occurred frequently in FRDA: there was a statistically significant reduction in the macular and foveal thickness, as well as in the average peripapillary RNFL thickness and in all but the temporal sectors in patient's group in comparison to controls. There have been very few prior OCT studies in FRDA and the number of patients evaluated in this cohort compares to the two largest groups reported to date [34, 101]. Although only a small proportion of individuals with FRDA were visually symptomatic, I found that majority had evidence of an underlying optic neuropathy detected by OCT. The average RNFL thickness in this cohort was $88.4 \pm 12.9 \mu\text{m}$, below the fifth percentile for age-matched controls ($103.9 \pm 8.4 \mu\text{m}$). Thus, this study shows that the visual pathway is affected in FRDA, and supports the hypothesis that retinal changes in FRDA are frequent.

The peripapillary RNFL thickness of this FRDA cohort was markedly thinner than the RNFL thickness in other genetically confirmed ataxia subtypes evaluated in this research (Chapters 6, 7, 8, 9, 10, and 11). The pattern of RNFL loss in FRDA is characteristic and, in contrast to other nuclear mitochondrial disorders where the temporal quadrant RNFL is most affected, in this FRDA cohort the temporal RNFL thickness was least reduced [212]. Interestingly, 44% of individuals had superior quadrant involvement, reported as the predominant sector affected in FRDA only in one prior study [34], while almost a third of patients exhibited inferior RNFL loss, and only a small proportion of individuals had RNFL thickness below the fifth percentile in remaining quadrants. Although abnormal RNFL thickness has been documented in other spastic ataxias associated with peripheral neuropathy, contrary to FRDA a distinct thickening of the RNFL is seen in ARSACS [125]. Thus, the results of this study support the hypothesis that OCT may play a role in distinguishing FRDA from other types of ataxias.

There was no significant association between RNFL thickness and disease duration, observed in some OCT studies in other FRDA cohorts [34, 99, 101]. Thus, my results suggest that RNFL changes as an anatomical marker are unrelated to disease duration in individuals with FRDA.

There was a correlation between RNFL parameters and neurological function and disability as measured with SARA score, showing that as cerebellar function declines, the RNFL becomes thinner. This particular relationship was previously assessed in only two other studies [100, 101], while other groups investigated RNFL thickness association with the more extensive ICARS and FARS, and similarly to the findings in this study, majority found a correlation between RNFL measurements as an anatomical marker and disease severity as quantified with clinical scales [34, 96, 97]. Overall, these findings suggest that OCT measurements and in particular RNFL thickness might be a useful tool for assessment of disease progression in FRDA patients, and thus, indicate that RNFL structural changes have a functional correlate.

Follow-up assessment of 20 individuals revealed significant decline in most OCT parameters: 95% of patients evaluated had average RNFL thickness decline, observed simultaneously with disability progression in 19/20 patients. Previously, only one small study of 8 FRDA patients reported macular and peripapillary RNFL thicknesses decline over the period of 6 months [100]. This study is the first large prospective study of its kind to provide positive outcomes, confirming that RNFL thickness declines over time and thus, retinal thickness as measured by OCT may be a candidate biomarker for disease progression in FRDA.

The results of this study provide important information regarding OCT findings in FRDA. This study highlights that FRDA is associated with frequent subclinical optic neuropathy and demonstrates that RNFL thickness as measured by OCT has the potential to become a

quantifiable biomarker for the evaluation of disease progression. Furthermore, the longitudinal data showing significant progression rates of retinal damage detectable through OCT support the usefulness of this cost-effective technique as an objective tool in future therapeutic trials.

12.2 Optical coherence tomography in *SPG7* - related spastic ataxia

In Chapter 6, I demonstrated that optic neuropathy is common in *SPG7* – associated spastic ataxia: although the average peripapillary RNFL was not statistically different from controls ($p=0.6$), RNFL thickness in the temporal quadrant was reduced in the patient group, compared to controls ($p<0.05$), and 20% of individuals had temporal RNFL thickness below the fifth percentile of normal, defined in the normative database provided by the manufacturer. This is the largest cohort description ($n=32$) of the peripapillary RNFL measures as detected by OCT in this entity; there have been only 16 previously documented *SPG7* cases to undergo OCT evaluation [130, 131, 132, 232] with optic neuropathy detected in 15 patients. Although only 6% of patients studied here had optic nerve atrophy, recognised as a disc pallor on fundus examination, I found that notable proportion had evidence of an underlying optic neuropathy detected by OCT. The temporal RNFL thickness in this cohort was $70.3 \pm 10 \mu\text{m}$, significantly reduced in comparison to age-matched controls ($75.2 \pm 9 \mu\text{m}$). Thus, this study shows that RNFL thickness is abnormal (predominantly in the temporal quadrant) in *SPG7* – associated spastic ataxia, and supports the hypothesis that retinal changes in *SPG7* are frequent.

The pattern of RNFL loss in *SPG7*–associated spastic ataxia demonstrates predominant temporal quadrant RNFL thinning, contrary to the distinct thickening of the RNFL seen in ARSACS, another AR spastic ataxia [91]. Furthermore, OCT findings in FRDA, an entity in which mitochondrial dysfunction has a central role, show predominantly average and superior quadrant RNFL loss, implying that optic neuropathy in FRDA is likely to involve

different disease mechanisms leading to diverse areas of selective vulnerability [34, 101]. Predominantly temporal RNFL reduction was also reported in one SCA1 study [105], however, this is a typically early-adulthood onset ADCA. Furthermore, these OCT findings were not replicated in another SCA1 study [38]. Overall, predominant abnormal RNFL thinning in the temporal sector seems to be a more consistent finding in *SPG7* cohorts [130, 131, 232]. Thus, the results of this study support the hypothesis that OCT may play a role in distinguishing *SPG7* – associated spastic ataxia from other types of ataxias.

There was no significant association between RNFL thickness and disease duration, similarly to previously reported heterogeneous HSP cohort with only 3 *SPG7* cases [130], and contrary to another genetically heterogeneous HSP cohort, which reported only 2 cases with *SPG7*-associated spastic ataxia. Thus, these results suggest that RNFL changes as an anatomical marker are unrelated to disease duration in individuals with *SPG7*.

There was no significant association between baseline RNFL thickness parameters and disease severity as quantified by SARA score. This particular relationship has not been previously assessed and thus, this study is the first to determine if such a correlation can be found. Similarly to my results, in series of patients with heterogeneous HSP, no correlation was found between RNFL thickness and disease severity assessed by SPRS [130, 132]. While SARA evaluates the ataxic manifestations, the SPRS quantifies the functional impairment of spastic paraplegia, suggesting that both scales have limitations in objectively estimating progression in the broader phenotypic spectrum of *SPG7*. Thus, these results suggest that RNFL changes in *SPG7* as an anatomical marker are unrelated to currently available clinical scales.

Follow-up assessment of 14 affected individuals revealed significant decline in RNFL thickness over time: 71% of patients evaluated had average and temporal RNFL thickness decline, observed simultaneously with disability progression in 11/14 patients. To my

knowledge, there have been no other studies evaluating progressive RNFL thickness changes in *SPG7* cohorts. Only one prior small longitudinal study reported OCT findings in a heterogeneous HSP cohort (n=9) and documented no significant RNFL thickness change compared to the baseline data for the period of observation, which was much shorter than in this study (mean 10.7 months _{HSP cohort} vs 20.1 months _{SPG7 cohort}) [132]. Thus, this study is the first prospective study of its kind to provide positive outcomes, confirming that RNFL thickness in paraplegin-related disease changes over time and thus, retinal thickness as measured by OCT may be a useful biomarker of disease progression in *SPG7*-associated spastic ataxia.

This study provides retinal data to help refine the phenotype of *SPG7*-related disease – an increasingly recognised spastic ataxia. RNFL abnormalities in the temporal quadrant, despite clinical heterogeneity, are common even in the absence of decreased visual acuity, and OCT should be considered part of the routine evaluation in HSP and CA with further work being required to clarify its value as a potential biomarker of disease progression in *SPG7*-associated disease.

12.3 Optical coherence tomography in other genetic and suspected genetic ataxias

In Chapter 7, I demonstrated that among the group of ADCA, retinal abnormalities are common in SCA7: affected individuals had noticeable degree of foveal and macular thinning with the macular thickness being the lowest among individuals with ADCA (mean $217.35 \pm 37.3 \mu\text{m}$) and visibly reduced when compared to healthy controls (mean $270.92 \pm 12.7 \mu\text{m}$). In contrast to prior larger studies, where normal average RNFL thickness has been reported in most patients [115], I found thinning of the RNFL with predominant changes in the superior more than inferior quadrant. I found that optic neuropathy is part of the phenotype in *SAMD9L*-associated ataxia pancytopenia syndrome, extending the previous knowledge about the associated phenotype [314]. My results demonstrated that optic neuropathy is not common in SCA1, 2, 3, 6, 14, and EA2, and although the average RNFL thickness in the SCA11 patient was within the normal range, temporal RNFL quadrant was thinner in comparison to other ADCA cases and controls. The macular parameters were reduced in SCA1 and SCA6 cases, and in contrast to prior larger study [38], I also found reduced macular thickness in SCA2.

In Chapter 8, I demonstrated that abnormal RNFL thickening is characteristic in ARSACS: average RNFL thickness in individuals with ARSACS was above 95% upper limit of normal, defined in the normative database provided by the manufacturer. This unique finding was not seen either in the control group, or in any of the other genetic ataxia cases.

In Chapter 9, I demonstrated that optic neuropathy is part of the phenotype in *DDHD2*- and *ANO10*-associated spastic ataxias, describing this previously not reported feature. Within the group of ARCA/ARHSP optic neuropathy was not detected in individuals with AOA1 and CANVAS.

In Chapters 10 and 11, I demonstrated that optic neuropathy is not a feature of *AIFM1*- and *MT-ATP6*-associated phenotypes.

Altogether, these studies show that visual pathway is affected in majority of the spastic ataxic syndromes and in SCA7, and thus, support the hypothesis that retinal changes are frequent in some but not in all subtypes of CA.

The pattern of RNFL loss in SCA7 demonstrates predominant superior and inferior quadrant thinning, while the temporal RNFL thickness measurements in the SCA7 patients were among the three lowest in the ADCA group. Although the average RNFL thickness was within the normal range, RNFL thickness in the temporal sector was reduced in the SCA11 case, compared to other ADCA and healthy controls, which was previously reported in some SCA1 cases [105]. Among the group of spastic ataxias, in addition to the reduced average RNFL thickness, abnormal thinning was seen across the sectors with the lowest temporal RNFL measurements documented in an individual with *DDHD2*-associated phenotype, while a tendency to predominantly superior quadrant thinning was found in *ANO10*-associated presentation, similarly to observations in the FRDA cohort, where 44% of individuals had superior quadrant involvement. In contrast to all of the above, distinct thickening of the RNFL is seen in ARSACS. Thus, the results of these studies support the hypothesis that OCT may play a role in distinguishing ARSACS from other types of spastic ataxia, and also SCA7 from other SCAs.

There was no significant association between RNFL parameters and ataxia severity as measured with SARA score in the cohort of individuals with *AIFM1*-associated phenotype. Thus, these results suggest that RNFL changes as an anatomical marker are unrelated to the severity of the ataxic disease component in individuals with mutations in the *AIFM1* gene.

Follow-up assessment of individuals with SCA1 and SCA3 demonstrated a tendency for RNFL thickness reduction in SCA1, but not in SCA3, simultaneously with disability progression in the former, not observed in the latter case. In contrast, a mild trend to RNFL

thickness decrease was seen in 5/8 eyes of patients with SCA3 [109]. Follow-up evaluation of a patient with ARSACS revealed a tendency to increased average macular and foveal thickness, while average RNFL thickness remained stable, and no disability progression as quantified with SARA score was observed. In contrast, follow-up assessment of one individual with mutation in the mitochondrial *MT-ATP6* gene showed no change in the OCT parameters, but disease progression over time was observed. This study is the first prospective study of individuals with SCA1, ARSACS and *MT-ATP6* – associated disease, confirming that RNFL thickness has a tendency to decline in SCA1 and increase in ARSACS over time and thus, retinal thickness as measured by OCT may be a useful biomarker of disease progression in these entities.

My data support the importance of obtaining OCT in all patients with different types of ADCA, ARCA and HSP, and particularly in those with reduced vision, as macular pathology and subtle optic neuropathy cannot be always reliably detected on funduscopy examination. Furthermore, my results suggest that different subtypes of ARCA / ARHSP exhibit distinct pattern of retinal thinning, thus supporting the importance of obtaining OCT especially in patients presenting with spastic ataxia.

12.4 Future directions

Despite evaluating a large cohort of individuals with CA, this study has some sample size limitations, particularly in the non-FRDA / non-SPG7 groups. Inherited CA are rare disorders and although the evidence suggests common optic nerve involvement, in some of the rarer subtypes of CA the number of individuals assessed were too small, and OCT results should be interpreted cautiously. Thus, further larger studies are needed to confirm that the findings from this study are reproducible, and to allow more precise identification of the specific retinal changes distribution.

As this is the first longitudinal study of retinal changes detected by OCT in some of the CA subtypes evaluated, further work is required in order to evaluate in greater details how optic neuropathy, seen in some of the CA subgroups, advances over time, to establish the rate of progression and its association with RNFL thinning. Ideally, larger patient cohorts should be examined that might help to identify which RNFL sectors are the best biomarkers of disease progression in different types of CA. A more systematic longitudinal approach in assessing the RNFL thickness is required also in order to help aggregate data from different studies and establish if there are specific subgroups of patients to be selected, and what the appropriate time-frame for follow-up assessment is.

To explore further the usefulness of OCT as a biomarker for disease progression in individuals with CA with detectable retinal changes on OCT, routine assessment with OCT of all individuals presenting with ataxia and particularly those with spastic ataxic phenotypes even in the absence of funduscopy changes should be considered.

Appendix 1. APOSTIL recommendations for reporting OCT results

Item	Recommendation	
1	Study protocol	Describe how many OCT operating sites and graders were included ☐
		Report the timing of OCT compared to other measurements (same day, delayed) ☐
		Describe the inclusion and exclusion criteria ☐
2	Acquisition device	For all OCT devices used, report data on:
		Manufacturer ☐
		Model ☐
		Version ☐
3	Acquisition Settings	Software version ☐
		Clearly describe the settings in which OCT scans were obtained:
		Room light conditions ☐
		Pupils dilated before examination (y/n) ☐
4	Scanning protocol	Number of operators and devices ☐
		Clearly describe the scanning protocol, including:
		Type of scan (circular, volume, star, line, other) ☐
		Location (area of interest, macula, optic nerve head, papillomacular bundle, other?) ☐
		Scan parameters (with or without eye tracking) ☐
		Volume scan: size of scan area (degrees or millimeters), number of B-scans, alignment of B-scans, number of A-scans per B-scan
		Radial scan: size of scan area (degrees or millimeters), number of B-scans, alignment of B-scans, number of A-scans per B-scan
5	Funduscopy imaging	Ring scan: diameter, A-scans/B-scan, manual or automatic placement of ring or method of centering, depth resolution
		Line scan: angle, location, number of A-scans, depth resolution
		Report other imaging modalities used in addition to OCT (funduscopy, confocal scanning laser ophthalmoscopy, retinal angiography, autofluorescence imaging) ☐
		Describe acquisition protocol, including: ☐
		Excitation wavelength ☐
		Filter sets ☐
6	Postacquisition data selection	Number of frames averaged (if applicable) ☐
		Describe image selection process, including: ☐
		Quality control criteria (i.e., OSCAR-IB ¹⁷ or other criteria) ☐
		Postacquisition discard (number and criteria) ☐
7	Postacquisition analysis	Eye selection strategy (if applicable) ☐
		Describe all postacquisition steps:
		Software used for processing scans and segmentation (may be different from acquisition software) ☐
		Which individual retinal layers were segmented/included ☐
		Method of segmentation (automated, semiautomated, or manually) ☐
		How potential bias was addressed in the case of manual segmentation (masking) ☐
8	Nomenclature and abbreviations	Grid used for data extraction (size, shape, selected sections) ☐
		Define:
		Anatomical structures analyzed ☐
9	Statistical approach	Units of provided measurements (e.g., volume or thickness) ☐
		Describe:
		Statistical models used for the analyses of OCT data ☐
		Whether data were analyzed by eye or by patient ☐

Abbreviation: OCT = optical coherence tomography.

Appendix 2. SCA Functional Index – Case Report Form

Handedness

Specify dominant hand:

- ☐ Dominant LEFT, non-dominant right
☐ Dominant RIGHT, non-dominant left

Timed walking test: 8m (25-foot) walk

8m walking test performed? ☐ yes ☐ no

If test has been performed

Assistive device: ☐ none
☐ one cane/crutch
☐ two canes/crutches
☐ wheeled walker
☐ orthosis

Did situations arise that necessitated repetition of a trial? _____

Other factors that might have affected performance? _____

Times are only given for 2 successfully completed trials:

Trial 1: _____ seconds (round to .1 second)

Trial 2: _____ seconds (round to .1 second)

Timed dexterity test: 9-hole peg test (9-HPT)

9-hole peg test performed? ☐ yes ☐ no

If test has been performed

Did situations arise that necessitated repetition of a trial? _____

Other factors that might have affected performance? _____

Dominant hand:

Trial 1: _____ seconds (round to .1 second)

Trial 2: _____ seconds (round to .1 second)

Non-dominant hand:

Trial 1: _____ seconds (round to .1 second)

Trial 2: _____ seconds (round to .1 second)

Timed speech task: PATA rate

PATA rate test performed? ☐ yes ☐ no

If test has been performed

Did situations arise that necessitated repetition of a trial? _____

Other factors that might have affected performance? _____

Counts are only given for 2 successfully completed trials:

Trial 1: _____ times

Trial 2: _____ times

Appendix 3. Scale for the Assessment and Rating of Ataxia

1) Gait Proband is asked (1) to walk at a safe distance parallel to a wall including a half-turn (turn around to face the opposite direction of gait) and (2) to walk in tandem (heels to toes) without support. 0 Normal, no difficulties in walking, turning and walking tandem (up to one misstep allowed) 1 Slight difficulties, only visible when walking 10 consecutive steps in tandem 2 Clearly abnormal, tandem walking >10 steps not possible 3 Considerable staggering, difficulties in half-turn, but without support 4 Marked staggering, intermittent support of the wall required 5 Severe staggering, permanent support of one stick or light support by one arm required 6 Walking > 10 m only with strong support (two special sticks or stroller or accompanying person) 7 Walking < 10 m only with strong support (two special sticks or stroller or accompanying person) 8 Unable to walk, even supported	2) Stance Proband is asked to stand (1) in natural position, (2) with feet together in parallel (big toes touching each other) and (3) in tandem (both feet on one line, no space between heel and toe). Proband does not wear shoes, eyes are open. For each condition, three trials are allowed. Best trial is rated. 0 Normal, able to stand in tandem for > 10 s 1 Able to stand with feet together without sway, but not in tandem for > 10s 2 Able to stand with feet together for > 10 s, but only with sway 3 Able to stand for > 10 s without support in natural position, but not with feet together 4 Able to stand for >10 s in natural position only with intermittent support 5 Able to stand >10 s in natural position only with constant support of one arm 6 Unable to stand for >10 s even with constant support of one arm
Score	Score
3) Sitting Proband is asked to sit on an examination bed without support of feet, eyes open and arms outstretched to the front. 0 Normal, no difficulties sitting >10 sec 1 Slight difficulties, intermittent sway 2 Constant sway, but able to sit > 10 s without support 3 Able to sit for > 10 s only with intermittent support 4 Unable to sit for >10 s without continuous support	4) Speech disturbance Speech is assessed during normal conversation. 0 Normal 1 Suggestion of speech disturbance 2 Impaired speech, but easy to understand 3 Occasional words difficult to understand 4 Many words difficult to understand 5 Only single words understandable 6 Speech unintelligible / anarthria
Score	Score

5) Finger chase Rated separately for each side Proband sits comfortably. If necessary, support of feet and trunk is allowed. Examiner sits in front of proband and performs 5 consecutive sudden and fast pointing movements in unpredictable directions in a frontal plane, at about 50 % of proband's reach. Movements have an amplitude of 30 cm and a frequency of 1 movement every 2 s. Proband is asked to follow the movements with his index finger, as fast and precisely as possible. Average performance of last 3 movements is rated. 0 No dysmetria 1 Dysmetria, under/ overshooting target <5 cm 2 Dysmetria, under/ overshooting target < 15 cm 3 Dysmetria, under/ overshooting target > 15 cm 4 Unable to perform 5 pointing movements			6) Nose-finger test Rated separately for each side Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to point repeatedly with his index finger from his nose to examiner's finger which is in front of the proband at about 90 % of proband's reach. Movements are performed at moderate speed. Average performance of movements is rated according to the amplitude of the kinetic tremor. 0 No tremor 1 Tremor with an amplitude < 2 cm 2 Tremor with an amplitude < 5 cm 3 Tremor with an amplitude > 5 cm 4 Unable to perform 5 pointing movements		
Score	Right	Left	Score	Right	Left
mean of both sides (R+L)/2			mean of both sides (R+L)/2		
7) Fast alternating hand movements Rated separately for each side Proband sits comfortably. If necessary, support of feet and trunk is allowed. Proband is asked to perform 10 cycles of repetitive alternation of pro- and supinations of the hand on his/her thigh as fast and as precise as possible. Movement is demonstrated by examiner at a speed of approx. 10 cycles within 7 s. Exact times for movement execution have to be taken. 0 Normal, no irregularities (performs <10s) 1 Slightly irregular (performs <10s) 2 Clearly irregular, single movements difficult to distinguish or relevant interruptions, but performs <10s 3 Very irregular, single movements difficult to distinguish or relevant interruptions, performs >10s 4 Unable to complete 10 cycles			8) Heel-shin slide Rated separately for each side Proband lies on examination bed, without sight of his legs. Proband is asked to lift one leg, point with the heel to the opposite knee, slide down along the shin to the ankle, and lay the leg back on the examination bed. The task is performed 3 times. Slide-down movements should be performed within 1 s. If proband slides down without contact to shin in all three trials, rate 4. 0 Normal 1 Slightly abnormal, contact to shin maintained 2 Clearly abnormal, goes off shin up to 3 times during 3 cycles 3 Severely abnormal, goes off shin 4 or more times during 3 cycles 4 Unable to perform the task		
Score	Right	Left	Score	Right	Left
mean of both sides (R+L)/2			mean of both sides (R+L) / 2		

Appendix 4. Genes tested in patients attending the NAC using commercially available NGS panels[#]

Oxford Ataxia Panel¹ (62 patients: 4/62 had 91-gene NGS panel testing; 58/62 had 98-gene panel testing)	AAAS, AARS, ABCB7, ABHD12, ADCK3, AFG3L2, AMPD2, ANO10, AP1S2, APTX, ARSA, ATCAY, ATP1A3, ATP8A2, C10orf2, CACNA1A, CACNB4, CASK, CCDC88C, CHMP1A, CLN6, CLP1, COX20, CYP27A1, CYP2U1, DARS2, DDHD2, DNMT1, ELOVL5, EXOSC3, FGF14, FLVCR1, FOLR1, GBA2, GOSR2, GRID2, GRM1, HEXA, HEXB, ITPR1, KCNA1, KCNC3, KCND3, KCNJ10, KIAA0226, MRE11A, MTPAP, NPC1*, NPC2*, OPHN1, PCLO*, PDYN, PEX16, PIK3R5, PLA2G6, PMPCA*, PNKP*, PNPLA6, POLG, PRKCG, PRRT2, RARS2, RELN, RNF170, SACS, SEPSECS, SETX, SIL1, SLC1A3, SLC2A1, SLC9A6, SNX14*, SPG7, SPTBN2, SRD5A3, STUB1, SYNE1, SYT14, TDP1, TGM6, TMEM240, TPP1, TSEN2, TSEN34, TSEN54, TTBK2, TTC19, TUBB4A*, UBR4, UCHL1, VAMP1, VLDLR, VPS53, VRK1, WDR81, WFS1, WWOX, ZFYVE26*, ZNF592
CeGat Ataxia panel 102 genes² (10 patients)	ABCB7, ABHD12, AFG3L2, ANO10, APTX, ARSA, ATCAY, ATM, ATP1A3, ATP8A2, CA8, CACNA1A, CACNA1G, CACNB4, CAMTA1, CAPN1, CCDC88C, CLCN2, COA7, COQ8A, CP, CTBP1, CWF19L1, CYP27A1, DARS2, DDHD2, DNAJC5, DNMT1, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, ELOVL4, ELOVL5, FGF14, FLVCR1, FXN, GBA2, GFAP, GOSR2, GRID2, GRM1, HEXA, HEXB, HSD17B4, ITPR1, KCNA1, KCNC3, KCND3, KCNJ10, KIF1C, MARS2, MRE11, MTTP, NKX6-2, NPC1, NPC2, PDYN, PEX7, PHYH, PLA2G6, PMPCA, PNKP, PNPLA6, POLG, POLR3A, PRICKLE1, PRKCG, PRRT2, PUM1, RNF170, RNF216, SACS, SCN2A, SCYL1, SETX, SIL1, SLC1A3, SLC2A1, SNX14, SOD1, SPG7, SPTBN2, STUB1, SYNE1, TDP1, TDP2, TGM6, TMEM240, TPP1, TTBK2, TTC19, TTPA, TWNK, VAMP1, VLDLR, VPS13D, WDR81, WFS1, WWOX, XRCC1
CeGat HSP 58 genes² (4 patients)	ABCD1, AFG3L2, AIMP1, ALDH18A1, ALS2, AP4B1, AP4E1, AP4M1, AP4S1, AP5Z1, ARL6IP1, ATL1, B4GALNT1, BSCL2, C12orf65, CAPN1, CPT1C, CYP2U1, CYP7B1, DDHD1, DDHD2, DSTYK, ERLIN2, FA2H, FARS2, GALC, GBA2, HSPD1, KIDINS220, KIF1A, KIF1C, KIF5A, L1CAM, MAG, NIPA1, NKX6-2, NT5C2, PLA2G6, PLP1, PNPLA6, REEP1, REEP2, RTN2, SACS, SLC16A2, SOD1,

	<i>SPART, SPAST, SPG11, SPG21, SPG7, TECPR2, TFG, TUBB4A, UBAP1, UCHL1, WASHC5, ZFYVE26</i>
Sheffield Ataxia panel 42 genes³ (2 patients)	<i>ABCB7, AFG3L2, APTX, ATM, ATP1A2, ATP1A3, ATP7B, C10orf2, CACNA1A, CACNB4, CYP2U1, CYP27A1, DDHD2, EEF2, FGF14, FTL, FXN, GBA, GBA2, IFRD1, ITPR1, KCNA1, KCNC3, KCND3, MTPAP, PDYN, PRKCG, PRRT2, SACS, SCN1A, SETX, SIL1, SLC16A2, SLC1A3, SLC2A1, SPG7, SPTBN2, TGM6, TTBK2, TTPA, VAMP1, ZFYVE26</i>
Sheffield HSP panel 42 genes³ (6 patients)	<i>AFG3L2, ALS2, AP5Z1, ATL1, B4GALNT1, BSCL2, C12orf65, CYP27A1, CYP2U1, CYP7B1, DDHD1, DDHD2, FA2H, FIG4, GBA2, GCH1, HSPD1, KIAA0196, KIF1A, KIF5A, L1CAM, MTPAP, NIPA1, PLP1, PSEN1, REEP1, REEP2, RTN2, SACS, SIGMAR1, SLC16A2, SLC2A1, SPAST, SPG11, SPG20, SPG21, SPG7, VAMP1, VPS37A, WDR45, ZFYVE26, ZFYVE27</i>
Liverpool HSP panel 21 genes⁴ (2 patients)	<i>ATL1, BSCL2, CYP7B1, FA2H, GJC2, HSPD1, KIAA0196, KIF5A, L1CAM, NIPA1, PLP1, PNPLA6, REEP1, SLC33A1, SPAST, SPG11, SPG20, SPG21, SPG7, ZFYVE26, ZFYVE27</i>
CeGat Episodic Ataxia panel² 4 genes (1 patient)	<i>CACNA1A, CACNB4, KCNA1, SLC1A3</i>
Centogene Episodic Ataxia panel⁵ 4 genes (1 patient)	<i>CACNA1A, CACNB4, KCNA1, SLC1A3</i>

#Genetic laboratories update the list of target genes regularly

*Genes added to the 91 gene panel and included in the 98 gene panel

^ *RELN* removed from the 98 gene panel

¹ www.ouh.nhs.uk/geneticslab

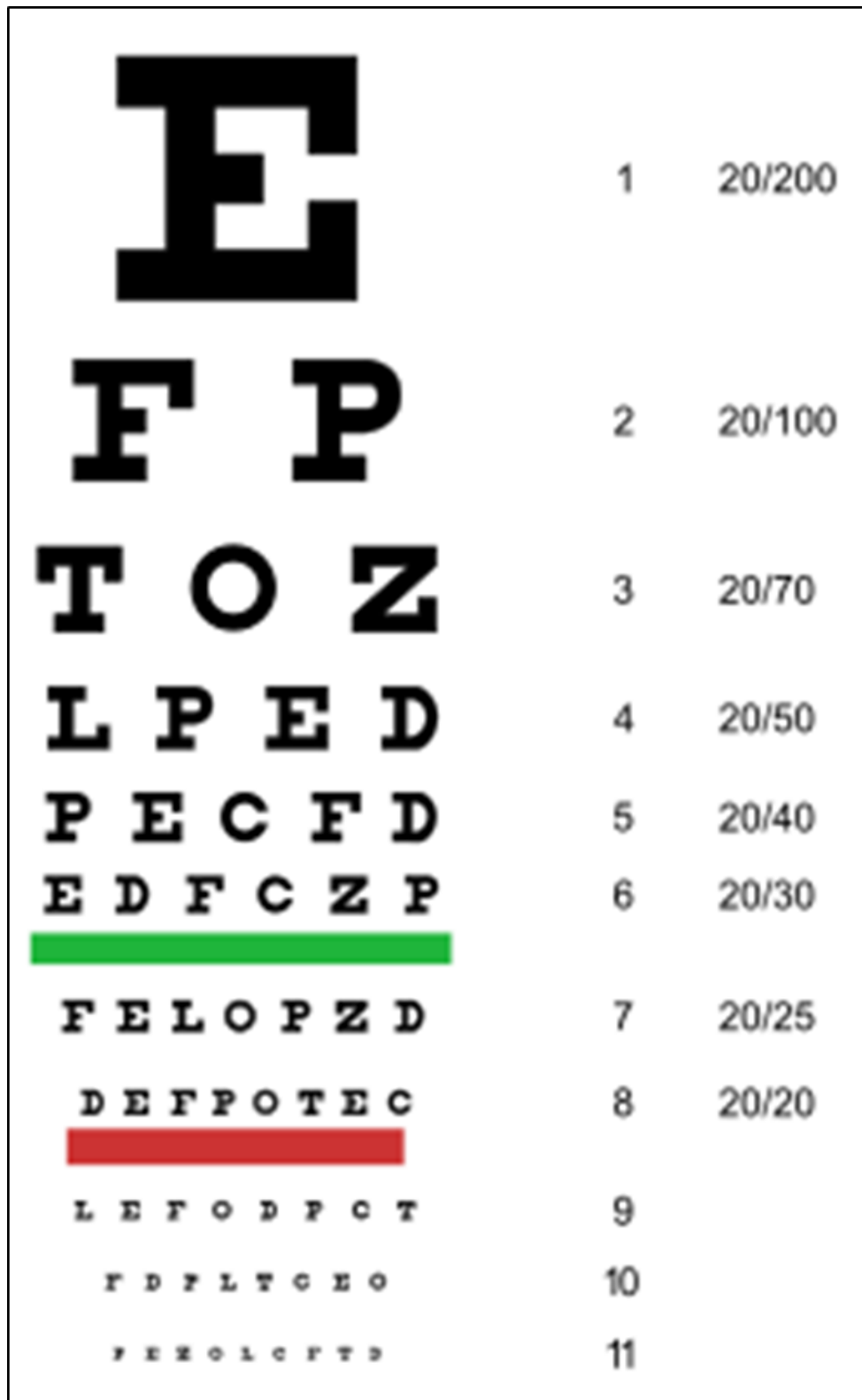
² www.cegat.de/en/diagnostics

³ www.sheffieldchildrens.nhs.uk/sdgs

⁴ www.liverpoolwomens.nhs.uk/health-professionals

⁵ www.centogene.com

Appendix 5. Snellen chart used to measure visual acuity



Appendix 6. Patient Information Leaflet

Title: Genotype phenotype study of rare inherited neurological disorders

What you should know about this study

You are being asked to take part in a study of rare inherited neurological disorders. This information form explains the project and your part in the study. Please read this form carefully. It tells you what you need to know about the study.

What is the purpose of the study?

The purpose of this study is to identify patients and families with rare neurological disorders (those affecting less than 1/20,000 people) with and without genetically confirmed diagnosis. These patients will undergo detailed clinical assessment and, if considered suitable, genetic testing. The information obtained will potentially help to reach a clinical and genetic diagnosis. In addition, this study aims to use clinical tools such as nerve conduction studies (NCS) and ocular coherence tomography (OCT) to investigate clinical phenotypes associated with rare genetically determined conditions.

People suitable to take part in the study are:

- Patients with rare inherited neurological disorders;
- Affected family members of a patient with a rare inherited neurological disorder;
- Unaffected family members of a patient with a rare inherited neurological disorder.

What does this study involve?

At your first visit, you will be assessed for eligibility into this study, provide informed consent, and have demographic information taken.

A detailed neurological and family history will be taken and you will be examined. Further investigations considered medically necessary may be performed, e.g. nerve conduction studies, genetic testing. These tests are considered standard of care.

A non-invasive OCT imaging may be performed to visualize and analyse your retina, the light-sensitive tissue lining the back of the eye, as in some rare neurological disorders characteristic retinal changes may occur.

Genetic testing, including Next Generation Sequencing (NGS) methods, will be used to try to identify the genetic cause of your condition. In comparison to traditional techniques, NGS allows testing even when we do not know the causative gene. This technology is much more efficient and faster and has the ability to detect novel genes. It is more likely to achieve a genetic diagnosis when affected families are large and DNA from many affected and unaffected family members is available.

When you return to your doctor for any routine visit as part of your standard care, they will ask you how you are doing and conduct a routine clinic visit.

If a genetic diagnosis has been made all the implications will be discussed with you at your visit.

If initial results are negative, then further testing may be considered in the future, results of which will be discussed with you at a later visit.

How many people will be in this study?

We will enrol as many patients and families as possible to participate in this study.

What are the possible risks of the study?

If we fail to make a genetic diagnosis using new genetic technology, you may be disappointed or frustrated at the lack of a confirmed diagnosis.

There is a risk of loss of privacy. There may also be risks involved from taking part in this study that are not known to researchers at this time. If new risks are determined during the course of the study, you will be made aware of these, as they may affect your willingness to continue participating.

There is a very small possibility of complications arising from blood testing and these include infection, bruising, excessive bleeding, haematoma, dizziness and fainting.

Incidental findings:

Using NGS methods, we may identify different genetic variants that could cause disorders other than the one you (or your child) are known to have. These are known as “incidental findings”. On average, their number is small and the majority of them are non-neurological, e.g. genes that increase the risk of developing cancer or cardiac disorders. Some of the incidental findings predict a risk of developing severe disorders that can occur during childhood. All incidental findings will be discussed on a case-by-case basis by the research team. If your child is found to have an incidental finding actionable in childhood according to the list recommended by the American College of Medical Genetics, you will have an option to be advised about the result and relevant management offered. If your child is found to have an incidental finding actionable in adulthood, they will have the option of being informed of this when they reach 18 years of age. In some cases, an incidental finding may be found in your child which has implications for one of their parents; in this case you will be given the option of receiving feedback. You will also be given the option of receiving feedback about any incidental findings which are actionable in adulthood which are found in you.

Are there benefits to being in the registry study?

If a genetic diagnosis has been achieved this will allow genetic counselling for you and genetic screening for your family. If a novel mutation has been detected this may benefit other people with similar health issues, now or in the future. If a genetic diagnosis is made, further unnecessary investigations will be avoided. In the future, achieving a genetic diagnosis may allow specific treatment, although such treatments are not currently available for the majority of rare inherited neurological disorders at this time.

Will taking part in this study cost me anything?

There are no costs to you for participating in this study.

What are your options if you do not want to join the study?

Your alternative to participating in this study is to not participate.

If you do not participate in this study, your regular medical care will not be affected.

If visual and/or audio recordings will be used, will I have the opportunity to review and edit these?

You have the right, should you wish, to review and edit any transcripts to which you have contributed.

Data protection

We will be using your personal information in our study to help us collect information about rare genetic neurological disorders. We will be processing your data according to the Article 6 and Article 9 of GDPR.

For the purposes of this study we will be collecting personal data, such as your name and date of birth, as well as medical data, including your medical history, family tree, neurological examination, various investigations data (e.g. nerve conduction studies, lumbar puncture, optical coherence tomography, etc) and genetic data.

Your doctor /study investigator and the research team conducting this study will have access to your medical records as per patients' standard care. Your personal data will be processed only as is necessary for the purposes of genotype phenotype correlation and will not be processed in any way that damage or stress will be caused to you. The Ethics Committee and any party to whom we are under a legal duty to disclose your data may review your records and will see information which can identify you.

Your medical data will be stored in a secure place with restricted access in Tallaght Hospital and will not leave the site.

Your personal information collected for this research will be kept as long as it is needed to conduct this research. Once your participation in the research is over, your information will be stored in accordance with applicable policies and regulations.

You are free to only answer questions that you want to answer. If you decide to take part in the study you can later change your mind and withdraw from the study. You are free to withdraw from participation in this study at any time. You will not suffer any penalty or lose any benefits if you decide not to take part in the study.

Under data protection law you have a right to request a copy of the personal data processed by the study researchers.

Under data protection law you have rights to restrict processing, to have any inaccurate information about you corrected, and to be forgotten, subject to certain exemptions.

Confidentiality

All information collected about you during the course of this project will be kept confidential to the extent permitted by law.

The clinical information collected in the study will be stored in a protected PC and protected hard-drive in Tallaght Hospital.

Information that directly identifies you (like your name or address) will not be released without additional written permission from you.

The investigators conducting this study are committed to making the results of the research public through scientific presentations and publications of research articles. Information from this study may be used for research purposes and may be published; however, your name will not be used in any publication.

Compensation

Your doctors are covered by standard medical malpractice insurance. Nothing in this document restricts or curtails your rights.

Right to decline participation and/or to withdraw

Participation in this study is voluntary. You may choose not to take part in this study. If you decide to take part in the study you can later change your mind and withdraw from the study. If you decide to withdraw from participation in this registry, you will be able to do this by contacting Dr Sinéad Murphy, Dr Richard Walsh or Dr Petya Bogdanova-Mihaylova at:

Phone number 01 414 4061

Address: Department of Neurology, Tallaght University Hospital, Dublin 24, Ireland

You are free to only answer questions that you want to answer. You are free to withdraw from participation in this study at any time. You will not suffer any penalty or lose any benefits if you decide not to take part in the study.

The Principle Investigator of this study may end your participation in this study at any time with or without your consent.

Who do I contact?

If you have any questions about this study now or in the future, you may contact Dr Sinéad Murphy, Dr Richard Walsh or Dr Petya Bogdanova-Mihaylova

Phone number: 01 414 4061

Address: Department of Neurology, Tallaght University Hospital, Dublin 24, Ireland

Data Controllers: Tallaght University Hospital

Phone number: 01 414 2000

Address: Tallaght, Dublin 24, Ireland

Data Processor(s): Dr Sinéad Murphy, Dr Richard Walsh and Dr Petya Bogdanova-Mihaylova

Phone number: 01 414 4061

Address: Department of Neurology, Tallaght University Hospital, Dublin 24, Ireland

Data Protection Officer:

Email: dpo@tuh.ie

If you have questions about your rights as a research subject, please contact:

St James's Hospital/Tallaght University Hospital Joint Research Ethics Committee

Email: ResearchEthics@tuh.ie

Phone: 01-414 2199

Appendix 7. Informed Consent Form

Title: Genotype phenotype study of rare inherited neurological disorders

This consent form may contain words that you do not understand. Please ask the registry staff to explain any words or information that you do not understand.

I have read, or had read to me, the attached information sheet on the above study and have been given a copy to keep. I understand the purpose of this study, the procedures to be followed, the potential risks and the potential benefits.

YES ☐ NO ☐

The information has been fully explained to me and I have had an opportunity to ask questions, and my questions have been answered to my satisfaction. I also understand that no guarantee can be given about the possible results.

YES ☐ NO ☐

I agree to have an Optical Coherence Tomography (OCT) performed and researchers to take pictures of my retina and obtain more detailed information about my eye structures.

YES ☐ NO ☐

I give permission for my medical records to be looked at and information taken from them to be analysed in the strictest confidence by the relevant and responsible people from the TUH Neurology Research Group or from organisations supervising the research.

YES ☐ NO ☐

I agree to allow the researches use my information (personal data) as part of this study as outlined in the information leaflet. I have been told that all medical information / data pertaining to me will be protected by the principles of confidentiality and both national and EU data protection legislation.

YES ☐ NO ☐

I understand that I am free to withdraw from the study at any time without giving a reason and this will not affect my future medical care.

YES ☐ NO ☐

I understand that I will not benefit financially in any way if this research leads to the development of a new treatment or medical test.

YES ☐ NO ☐

I agree to be contacted by researches as part of this study.

I have been told how to contact the research team if I have additional questions.

YES ☐ NO ☐

I agree to be contacted in the future about potential studies I may be eligible for

YES ☐ NO ☐

I consent to take part in this research study having been fully informed of the risks, benefits and purpose of the registry

YES ☐ NO ☐

I give my explicit consent to have my data processed as part of this registry

YES ☐ NO ☐

Statement of Consent

I have read this consent form carefully and agree to participate in this study.

YES ☐ NO ☐

Printed name of Participant

Participant Signature

Date

Statement of Person Conducting Informed Consent Discussion

I have discussed the information contained in this document with the participant and it is my opinion that the participant understands the risks, benefits, alternatives and procedures involved with this research study.

YES ☐ NO ☐

I confirm that I have given a copy of the information leaflet and consent/assent form to the participant.

YES ☐ NO ☐

Printed name of person obtaining consent

Signature of person obtaining consent

Date

Assent (if applicable)

To be completed by the Child taking part in the study

- I have read and understood the Participant information YES ☐ NO ☐
- I have had the opportunity to discuss this study, ask questions about the study and I have received satisfactory answers to all my questions YES ☐ NO ☐
- I have received enough information about this registry YES ☐ NO ☐
- I agree to have an Optical Coherence Tomography (OCT) performed and researchers to take pictures of my retina and obtain more detailed information about my eye structures. YES ☐ NO ☐
- I understand that I am free to withdraw from the study at any time without giving a reason and without this affecting my future medical care YES ☐ NO ☐
- I agree to allow the researches use my information (personal data) as part of this study as outlined in the information leaflet YES ☐ NO ☐
- I agree to allow the researches access my medical records as part of this study YES ☐ NO ☐
- I agree to be contacted by researches as part of this study YES ☐ NO ☐
- I agree to have my data processed as part of this study YES ☐ NO ☐
- I have had the above research study explained to me in language I understand and I agree to participate. YES ☐ NO ☐
- I agree to be contacted in the future about studies that I may be eligible for YES ☐ NO ☐

Child's Name

Child Signature

Date

To be completed by the Parent / Legar Guardian of participant

- I consent for my child to take part in this research study having been fully informed of the risks, benefits and purpose of the study YES ☐ NO ☐
- I give my explicit consent to have my child's data processed as part of this research study YES ☐ NO ☐

Parent / Legal Guardian Name

Parent / Guardian Signature:

Date:

Statement of Person Conducting Informed Consent Discussion

I have discussed the information contained in this document with the participant and it is my opinion that the participant understands the risks, benefits, alternatives and procedures involved with this research study. YES ☐ NO ☐

I confirm that I have given a copy of the information leaflet and consent/assent form to the participant. YES ☐ NO ☐

Printed name of person obtaining consent

Signature of person obtaining consent

Date

Appendix 8. Nerve conduction studies normative values

Median Motor	Lat $\leq$ 4.0ms; Amp $\geq$ 5.0mV; CV $\geq$ 50m/s;
Tibial Motor	Lat $\leq$ 6.0ms; Amp $\geq$ 4.0mV; CV $\geq$ 40m/s;
Peroneal Motor	Lat $\leq$ 6.0ms; Amp $\geq$ 2.5mV; CV $\geq$ 40m/s;
Superficial Radial Sensory	Lat $\leq$ 2.9ms; Amp $\geq$ 16μV*; CV $\geq$ 50m/s;
Sensory Median	Lat $\leq$ 3.3ms; Amp $\geq$ 7.0μV; CV $\geq$ 50m/s;
Sensory Ulnar	Lat $\leq$ 3.1ms; Amp $\geq$ 5.0μV; CV $\geq$ 50m/s;
Sural	Lat $\leq$ 4.4ms; Amp $\geq$ 6.0μV*; CV $\geq$ 40m/s;

*Less in patients of advanced age

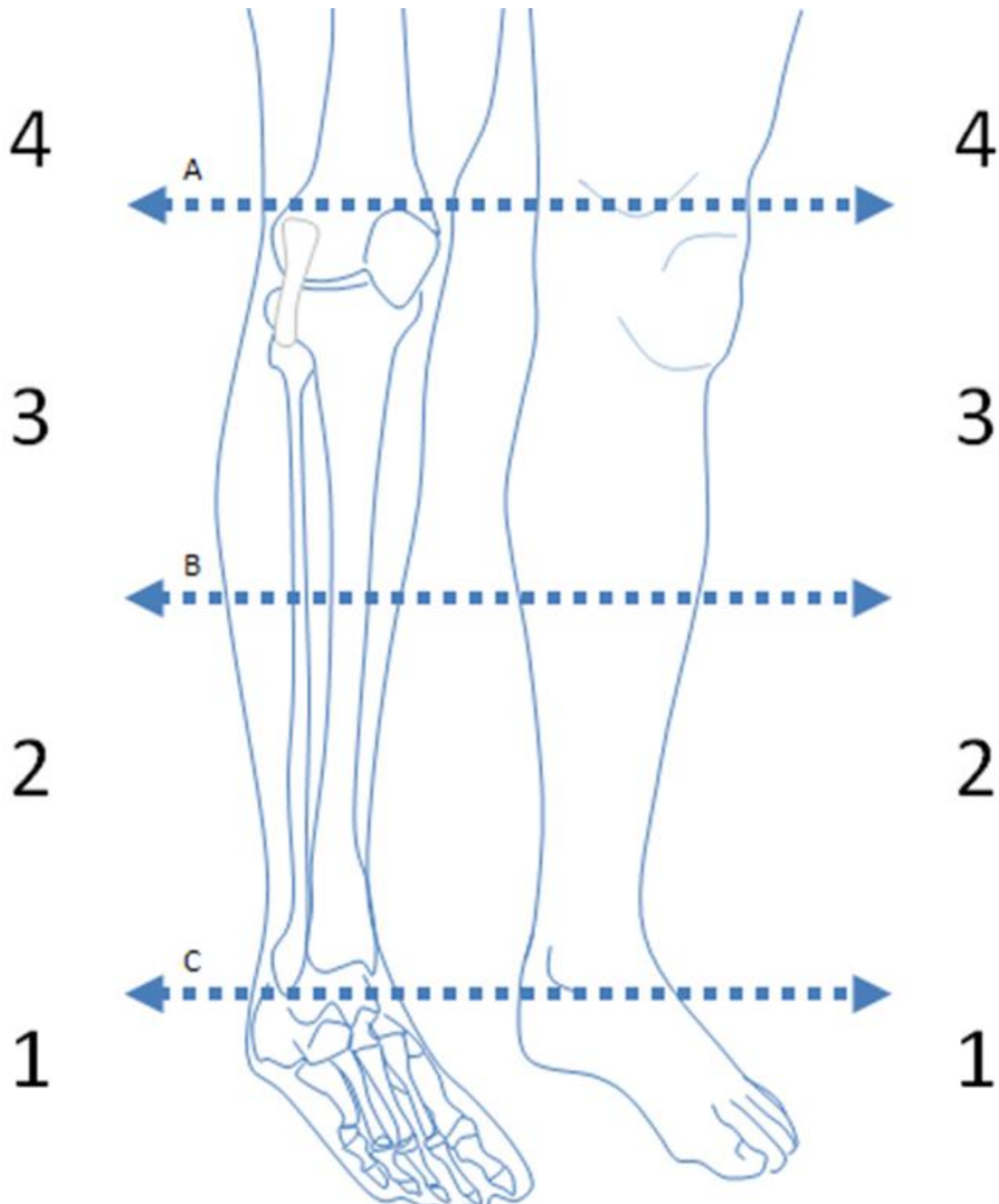
Median and Ulnar sensory nerves are examined orthodromically;

Superficial radial and Sural nerves are examined antidromically

Appendix 9. CMT Neuropathy Score – Version 2

Parameter	0	1	2	3	4	Score
Sensory symptoms ¹	None	Symptoms below or at ankle bones	Symptoms up to the distal half of the calf	Symptoms up to the proximal half of the calf, including knee	Symptoms above knee (above the top of the patella)	
Motor symptoms legs ²	None	Trips, catches toes, slaps feet. Shoe inserts	Ankle support or stabilization (AFOs). Foot surgery ⁵	Walking aids (cane, walker)	Wheelchair	
Motor symptoms arms	None	Mild difficulty with buttons	Severe difficulty or unable to do buttons	Unable to cut most foods	Proximal weakness (affect movements involving the elbow and above)	
Pinprick sensibility ^{1,3}	Normal	Decreased below or at ankle bones	Decreased up to the distal half of the calf	Decreased up to the proximal half of the calf, including knee	Decreased above knee (above the top of the patella)	
Vibration ⁴	Normal	Reduced at great toe	Reduced at ankle	Reduced at knee (tibial tuberosity)	Absent at knee and ankle	
Strength legs	Normal	4+, 4 or 4- on foot dorsiflexion or plantar flexion	≤ 3 on foot dorsiflexion or ≤ 3 on foot plantar flexion	≤ 3 on foot dorsi and ≤ 3 on plantar flexion	Proximal weakness	
Strength arms	Normal	4+, 4 or 4- on intrinsic hand muscles ⁵	≤ 3 on intrinsic hand muscles ⁶	< 5 on wrist extensors	Weak above elbow	
Ulnar CMAP (Median)	>6mV (>4mV)	4-5.9mV (2.8-3.9)	2-3.9 mV (1.2-2.7)	0.1-1.9 mV (0.1-1.1)	Absent (Absent)	
Radial SAP amplitude, antidromic	≥15μV	10 - 14.9 μV	5 - 9.9 μV	1 - 4.9 μV	< 1 μV	
CMTSS Subtotal						
CMTES Subtotal						
CMTNS Total						

Notes: 1: Use the picture below to discriminate the level of the symptoms; 2: Uses aid most of the time. The patient was prescribed to wear/use or should be wearing/using the aid in the examiner's opinion (see written instructions); 3: Abnormal if patient says it is definitely decreased compared to a normal reference point; 4: Use Rydell Seiffer tuning fork. Definition of Normal: > 5; 5: See written instructions for details of eligible foot surgery; 6: Intrinsic hand muscles strength assessment: Test only Abductor Pollicis Brevis (ABP) and First Dorsal Interosseus (FDI), then choose the stronger to give the score.



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