

Summary

Thesis title: Retinal thickness in adults with Down's syndrome: Relationship with age, cognition and dementia.

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People with Down's syndrome (DS) are known to experience premature ageing and have a high propensity for clinical diagnosis of dementia due to Alzheimer's disease (AD). In DS there is a unique and natural model of over-expression of amyloid-beta ($A\beta$) protein, the accumulation of which is proposed to be the central early event in the pathogenesis of AD. In DS, AD neuropathology is universally seen in the brain from the fourth decade. Identifying biomarkers are essential to the evaluation of future treatment trials. The retina has been shown to experience changes in patients with AD, such as retinal thinning, compared to age-matched controls. As an extension of the brain, the retina can be quickly and non-invasively imaged and may provide a proxy measure of brain changes in AD.

Using optical coherence tomography (OCT), cross-sectional retinal examinations were completed in 50 people with DS aged 18 years and over. Comparisons between retinal thickness of the DS and control groups were examined, as well as the effect of age on thickness in both groups. For the DS group, further investigations were made into the relationships between retinal thickness and (i) cognitive performance, (ii) diagnosis of dementia, (iii) cortical thickness and, (iv) presence of $A\beta$ binding in the brain.

Contrary to expectations, people with DS had thicker retina compared to age-matched controls. In addition, normal age-related retinal thinning was not seen in the DS group. People with DS have a life-long overproduction of $A\beta$, deposits of which have been previously imaged in the retina. $A\beta$ may be responsible both directly, through physical mass, and indirectly through inflammation as a response to $A\beta$, for increased retinal thickness in people with DS. Consequently, retinal thickness in DS may be a proxy measure of $A\beta$ deposition in the retina.

As part of a collaborative study, brain $A\beta$ binding was measured using positron emission tomography neuroimaging in a subset of the DS group. Individuals with positive Pittsburgh compound [11C]-PIB (PIB) binding to $A\beta$ displayed a trend towards having thinner retina than those with negative PIB binding. These results indicate that a shift towards thinning retina in people DS may reflect changes in brain pathology. Future studies are discussed which aim to investigate $A\beta$ and $A\beta$ driven pathology in the retina.

Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or any other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

This dissertation does not exceed the maximum permitted word limit of 60,000 words

Signed:

Madeleine Jane Walpert

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List of Abbreviations

µm	Micrometres
AD	Alzheimer's disease
AMD	Age-related macular degeneration
ANOVA	Analysis of variance
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
ART	Automatic retinal tracking
Aβ	Amyloid-beta
BACE	B-site amyloid precursor protein cleaving enzyme
BP _{ND}	Non-displaceable binding potential
CAMCOG	Cambridge Cognition Examination
CAMDEX	Cambridge Examination for Mental Disorders
CIDDRG	Cambridge Intellectual and Developmental Disabilities Research Group
CNS	Central nervous system
DARC	Detection of apoptosing retinal cells
DFT	Dementia of frontal type
DiDS	Dementia in Down's syndrome study
DS	Down's syndrome
DSA	Down's syndrome association
EEG	Electroencephalography
GCL	Ganglion cell layer
Heyex	Heidelberg eye explorer
ICORG	Imperial College Ophthalmic Research Group
ID	Intellectual disability
INL	Inner nuclear layer
IPL	Inner plexiform layer
KBIT-2	Kaufman Brief Intelligence Test, second edition
MOCA	The Montreal Cognitive Assessment
MCI	Mild cognitive impairment
MCP-1	Monocyte-chemotactic protein - 1
MMSE	Mini Mental State Examination
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
OCT	Optical coherence tomography

ONL	Outer nuclear layer
OPL	Outer plexiform layer
PET	Positron emission tomography
PIB	Pittsburgh compound [¹¹ C]
RNFL	Retinal nerve fibre layer
RPE	Retinal pigment epithelium
SD	Standard deviation
TICS	Telephone interview for cognitive screening
WBIC	Wolfson brain imaging centre

Chapter One:

Introduction

1.1 Objective

The aim of this study is to investigate retinal thickness in adults with Down's syndrome (DS) in relation to ageing and the onset of Alzheimer's disease (AD) using optical coherence tomography (OCT). People with DS have a very high prevalence of early-onset dementia of the Alzheimer's type, which manifests with the typical neuropathological features seen in typically developing AD, including amyloid-beta ($A\beta$) plaques, neurofibrillary tangles and neuronal death. DS is proposed as a unique and natural model through which AD pathology and mechanisms can be studied (Teipel & Hampel, 2006). Research in sporadic AD in the general population has found that retinal thickness measures can be used to successfully distinguish between patients with AD and age-matched controls, and that subtle changes in the retina could provide early markers of AD. In this study, retinal thickness was examined in adults with DS and compared to typically developing age-matched controls and an older comparison group. Thickness measures were evaluated in association with age, cognitive performance and dementia status. This cross-sectional study is the first to investigate retinal thickness in an adult population of DS and has examined the possibility that the retina could become a marker of AD in people with DS. Early identification of AD related change in the form of robust biomarkers is essential to the development of effective treatments that are aimed to slow or stop the progression of the disease. Retinal changes not only have the potential to be markers of early AD, but the retina could become a site to be used for measuring and monitoring the efficacy of future treatment trials.

1.2 Down's syndrome

1.2.1 Trisomy of chromosome 21

Down's syndrome (DS) was first described by John Langdon Down in 1866 (Down, 1866) as a set of characteristics identified in many of his patients with intellectual disabilities (ID). For almost 100 years the cause of the syndrome was unknown, until in 1959, Lejeune identified the trisomy of chromosome 21 (Lejeune 1959). Full trisomy of chromosome 21 accounts for approximately 95% of cases of people with DS, the remaining 5% include

mosaicism and partial trisomy caused by Robertsonian translocation (Hunter, 2010; Mutton, Alberman, & Hook, 1996). People with DS display particular phenotypical characteristics due to dysregulated expression of multiple genes (see section 1.2.2). In individuals with mosaicism and translocation the DS phenotype is almost always less pronounced. Although genes are significant in the manifestation of DS, abnormalities seen in patients are multifactorial and the result of combined genetic and environmental influences (Reeves, Baxter, & Richtsmeier, 2001). DS is the most common cause of ID worldwide, accounting for 10 in 10,000 and 14 in 10,000 live births in Europe and the United States respectively (Loane et al., 2013; Parker et al., 2010). Improved antenatal screening has increased the termination rate of DS pregnancies, leading to a 1% reduction in live births. Without antenatal screening it is predicted that DS births would have risen substantially in accordance with rising maternal age (Morris & Alberman, 2009). It is estimated that in England and Wales there are approximately 38,000 people with DS (Wu & Morris, 2013). The median life expectancy of a person with DS has increased dramatically in recent decades, from 12 years in the 1940s, to 60 years in 2002 within developed countries (Bittles & Glasson, 2004; Bittles, Bower, Hussain, & Glasson, 2007). Longer life expectancy is attributed to both improvements in health and social care and general awareness of DS.

Increased longevity has raised awareness of the issue of dementia in people DS. However, a lifespan of approximately 60 years falls drastically short of what is expected in developing countries for those without DS, and is most likely due to the high prevalence of early-onset AD (Holland, Hon, Huppert, Stevens, & Watson, 1998). Dementia research in people with DS is not only important for the DS population but also for AD in general. The similarities in the presentation of the disease, along with the over-expression of A β in DS mean that people with DS will likely be the first population for whom treatment trials show positive results, from which they would hopefully be generalised into sporadic AD.

1.2.2 Phenotype

People with DS share certain physical characteristics as a result of the dysregulated expression of some genes, these include; short stature, flat nasal bridge, small head, broad hands, small ears and mouth, and upward slanting eyes with epicanthic folds. These features are characteristic of DS, but are not homogeneous within the syndrome (Roizen & Patterson, 2003). From a cognitive perspective, all individuals with DS present with some level of ID,

again this varies significantly. Characteristically people with DS have slow development and cognitive impairment is seen in the following areas; phonological disorder in speech (Dodd & Thompson, 2001), verbal working memory (Baddeley & Jarrold, 2007) and short attention span (Dierssen, 2012; Lott & Dierssen, 2010). Individuals with DS present with a limited capacity short-term memory span (Purser & Jarrold, 2010) and deficits on long term memory tasks (Pennington et al., 2015). Typical IQ range is between 20 and 80 points (Nadel, 2003). There are many health conditions associated with the DS phenotype, most common are; congenital heart defects, hearing and vision impairments, thyroid dysfunction, childhood leukaemia, skin and dental abnormalities, seizures later in life, obesity and early-onset dementia (Freeman et al., 2008; Krinsky-McHale, Jenkins, Zigman, & Silverman, 2012; Roizen & Patterson, 2003). Research in adults with DS is relatively scarce in comparison to research conducted in infants and children, partly due to the relatively recent improvement in lifespan.

1.2.3 Accelerated ageing

People with DS appear to exhibit changes that are typically associated with old age much earlier in life (Oliver & Holland, 1986; Zigman, 2013). Signs of physical ageing such as skin thinning and wrinkling, greying and loss of hair is seen in individuals between the ages of 30 and 70 (Brugge, Grove, Clopton, Grove, & Piacquadio, 1993), and the autoimmune condition alopecia areata has been reported as commonly emerging in the third or fourth decade of life (Carter & Jegasothy, 1976). Motor skills in people with DS decline early in life, reaction time, grip strength and gait speed have all been found to correlate with age and begin to deteriorate from middle age (Smith, Stergiou, & Ulrich, 2005; Smith & Ulrich, 2008). Levels of physical activity in people with DS are reduced in comparison to those without DS, both typically developing controls and individuals with non-DS ID. Studies have shown found that no children or adults with DS met the recommended daily physical activity recommendations, and engaged in more sedentary behaviour than those without ID. Activity levels were found to further reduce alongside increasing age (Phillips & Holland, 2011). Lower levels of exercise may increase the risk of people with DS developing diseases associated with physical inactivity. People with DS are diagnosed with osteoporosis, hypothyroidism and vision and hearing complaints earlier in life, and women experience

early menopause (Baptista, Varela, & Sardinha, 2005; Carr & Hollins, 1995; Covelli, Raggi, Meucci, Paganelli, & Leonardi, 2016; Milberger et al., 2002; Zigman, 2013).

The most striking age-related change in people with DS is undoubtedly the early onset of dementia. Dementia manifests in clinical symptoms at around the age of 80 years in the typically developing population, in people with DS, age of dementia diagnosis is closer to 50 years. Pathological changes occur in the brain many years before clinical onset. AD neuropathology has been reported in the brains of virtually all people with DS over the age of 30 years (Wisniewski, Dalton, Mclachlan, Wen, & Wisniewski, 1985), including A β senile plaques, neurofibrillary tangles, brain atrophy and volume reduction, found in individuals from the age of 40 years onwards, regardless of a diagnosis of dementia or any sign of clinical symptoms (Annus et al., 2016; Beacher et al., 2010; Mann & Esiri, 1989; Nelson et al., 2011; Wisniewski et al., 1985). Brain changes in people with DS are similar to those seen in the brains of non-DS patients with sporadic, or late-onset AD, only much earlier in life (Jack et al., 2014).

1.2.4 Ocular disorders

The eyes of people with DS have particular characteristics, they are typically small, upward slanting and have epicanthic folds (Dykens, Hodapp, & Finucane, 2000; Scherbenske, Benson, Rotchford, & James, 1990). Ocular and orbital abnormalities are reported in people with DS, with varying frequencies. There is increased prevalence of nystagmus, strabismus, keratoconus, amblyopia, refractive error, astigmatism, hyperopia and cataracts in this population, most often reported in children (Da Cunha & Moreira, 1996; Kim, Hwang, Kim, & Yu, 2002; Merrick & Koslowe, 2001; Weiss, Kelly, & Phillips, 2016). In adults with DS, research is more scarce, however, adult population studies have also shown an increased prevalence of the ocular disorders seen in children (Evenhuis, Theunissen, Denkers, Verschuure, & Kemme, 2001; Krinsky-McHale et al., 2012). In adults, research has indicated an age-related increase in ocular disorders. Krinsky-McHale et al., (2012) reported a large increase from a 5.1% prevalence of an ophthalmic disorder in the thirties, to 41.3% prevalence in those in their forties. Evenhuis, Theunissen, Denkers, Verschuure, & Kemme, (2001) reported a smaller change from 4% in individuals under 50 years to 7.5% in the over fifties, although much higher prevalences (51% and 53% respectively) were seen in individuals with severe/profound ID compared to those with mild/moderate ID.

Incidence rates of ophthalmic diseases commonly associated with old age have been identified in younger people with DS, particularly cataracts, which are often assessed for at birth and seen in children (Cunha & Moreira, 1996). In adults, cataract severity was generally reported to be mild and corrective surgery was rare. In older individuals the highest cataract prevalence reported was 42%, with the average age of diagnosis being 48 years (Krinsky-McHale et al., 2012). Glaucoma is another age-related ocular disorder, however this is not so frequently reported in people with DS. In children, rates of primary childhood glaucoma are lower (<1%) than in typically developing children (2.29%; Aponte, Diehl, & Mohny, 2010; Fimiani et al., 2007; Stephen, Dickson, Kindley, Scott, & Charleton, 2007; Wong & Ho, 1997), whilst for adults reports are more varied, 2% in an age range of 30-83 years (Caputo, Wagner, Reynolds, Guo, & Goel, 1989) and 5% in an age range of 3.5 months to 26 years (Krinsky-McHale et al., 2012). In the typically developing population glaucoma prevalence is 3.54% between the ages of 40 and 80 years (Tham et al., 2014). Yokoyama found significantly higher rates of glaucoma in people with DS (11.5%) compared to age-matched controls (1.1%), in this study, glaucoma diagnosis was based on optic disc abnormalities, and it was noted that there was no difference in the intraocular pressure between glaucomatous and non-glaucomatous eyes in the DS group. It has been suggested that glaucoma could be over-diagnosed in people with DS, perhaps due to the similarities between glaucoma presentation and amyloid-related retinal pathology presentation (see Figure 1.6, section 1.4.3), and one of the crucial differences being that increased intraocular pressure is only seen in glaucoma.

1.3 Alzheimer's disease in people with Down's syndrome

1.3.1 Introduction

Alzheimer's disease is a degenerative neurological disorder and the most common cause of dementia, affecting an estimated 46.8 million people in the year 2015. This number is expected to double every 20 years with the ageing population, potentially reaching 131.5 million by 2050 (Mahajan & Votruba, 2017). The hallmarks of familial, or sporadic AD are chiefly; senile plaques containing A β peptide, neurofibrillary tangles consisting of hyperphosphorylated tau protein, and, neuronal death (Hardy & Selkoe, 2002). Expression of AD in people with DS is very similar to that of the typically developing population, the main difference being the age at which the pathology manifests. In the typically developing

population, dementia is usually diagnosed after the age of 80 years, although it is understood that AD pathology is present in the brain for many years, even decades prior to clinical presentation (Bateman et al., 2012). In children with DS, instances of neuritic A β plaques have been seen as young as eight years (Olson & Shaw, 1969), and are frequently seen in adults with DS from the age of 35 years (Burger & Vogel, 1973; Hof et al., 1995; Leverenz & Raskind, 1998; Wisniewski et al., 1985). After the age of 40, virtually all people with DS will express A β in the brain (Annus et al., 2016; Beacher et al., 2010; Mann & Esiri, 1989; Nelson et al., 2011; Wisniewski et al., 1985), and typical age of dementia onset is 50 years (Holland, Hon, Huppert, Stevens, & Watson, 1998; Oliver, Crayton, Holland, Hall, & Bradbury, 1998).

1.3.2 Prevalence

Despite that all individuals with DS present with AD pathology, not all develop the clinical presentation of dementia, as was thought to be the case until as recently as the 1980s. Prevalence rates of dementia in people with DS are varied, likely due to difficulties in the assessment of dementia in people with DS, (discussed in more detail in section 1.3.3), however, it is certain that the incidence of age-related cognitive decline and onset of dementia is considerably higher and occurs at a much younger age in people with DS compared to the typically developing population.

Variation in reported dementia prevalence in people with DS is vast (see Figure 1.1); Coppus et al., (2006) found that incidence rates doubled every five years from 49 years (8.9%) to 59 years (32.1%). Holland, Hon, Huppert, Stevens, & Watson, (1998) looked at a wider age range, finding relatively low prevalence in the thirties (3.4%), increasing to 10.3% in the forties and up to 40% in the 50+ age group. Tyrrell et al., (2001) found fewer incidences of dementia in people with DS than other research studies, with a prevalence rates of 5.7% in the 40-50 category and 30.4% in the 50-60 age group. In older individuals with DS prevalence records are even more diverse, from 25.6% in those in their 60s (Coppus et al., 2006), to 100% diagnosis in those over 70 years (Visser, Aldenkamp, Van Huffelen, & Kuilman, 1997). Lower prevalence rates are likely to be influenced by increased mortality rates in this age group, and it could be argued that those surviving past the age of 70 could be protected from AD.

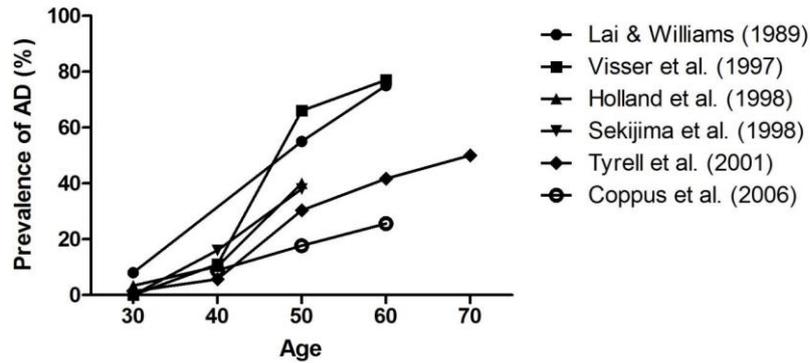


Figure 1.1 Graphical representation of the reported variation in age-specific prevalence of dementia in adults with DS across prevalence studies. Figure reproduced from Wilson, Annus, Zaman, & Holland, (2014).

1.3.3 Presentation and assessment

Early clinical presentation of dementia is different in people with DS than in typically developing patients (Holland, 2000; Holland et al., 1998). Rather than the classic symptoms of memory loss and forgetfulness, Holland and colleagues have found that changes in personality and behaviour, including social withdrawal, apathy, and stubbornness are among the first dementia-related changes that are seen in people with DS (Ball et al., 2006; Holland, Hon, Huppert, & Stevens, 2000; Holland et al., 1998). Ball et al., (2006) found that individuals identified as having personality and behaviour changes were more likely to have developed AD by a re-assessment five years later than those with changes in other areas of functioning. Behavioural and personality changes are commonly more related to the presentation of “dementia of the frontal type” (DFT), however progression of dementia in people with DS results in an eventual diagnosis of AD rather than DFT. Personality and behaviour are functionally associated with the frontal lobes which are developmentally smaller in people with DS (Pinter, Eliez, Schmitt, Capone, & Reiss, 2001) and may be compromised earlier in the AD pathway.

Diagnosing AD in people with DS is completed using the same pathway and diagnostic criteria as in the typically developing population, as is described in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013) and the tenth edition of the International Classification of Diseases (World Health Organisation, 1992). Whilst it is desirable to have consistency in the classification of AD, it

should be recognised that there are additional complexities when assessing dementia in a person with DS. The primary challenge is to determine the extent to which cognitive and functional limitations are a result of deterioration due to dementia, rather than relating to pre-existing ID, or due to effects of ageing (Holland and Walpert, 2016). Dementia assessments are usually undertaken when a friend, family member or carer of the individual with DS becomes concerned about their changing behaviour; however, this is not the optimal baseline assessment. Ideally, baseline assessments should be completed before there are any signs of change and routinely followed up over several years to establish a “cognitive profile” of the individual. Another difficulty in the diagnosis of AD in people with DS is the complexity of pseudo-dementia. Pseudo-dementia is a term for a condition that can present with similarities to dementia, but that is not a progressive disorder of the brain. The conditions include: severe depression, hypothyroidism, vitamin B12 and folate deficiencies, and brain tumours. Most commonly there is a misdiagnosis of AD in those with severe depression. This problem is not exclusive to people with DS, in fact, in DS it is argued that the pseudo-dementias are easier to eliminate because of the high incidence of early-onset AD – however this thought process may also lead to an over diagnosis of AD.

1.3.4 Mechanisms of Alzheimer’s disease

1.3.4.1 The amyloid-beta cascade hypothesis

Central to the theory of AD in people with DS is the trisomy of chromosome 21, which includes triplication of the amyloid precursor protein (APP) gene. APP is a precursor molecule whose proteolysis generates A β . Fibrillar A β is found in senile plaques in the brains of people with AD and is considered one of the driving forces and potentially the most influential pathology of AD, particularly in people with DS.

The amyloid cascade hypothesis, proposed by Hardy and Higgins in 1992, proposes that excessive build-up of A β protein in the brain is the main causative agent of the subsequent neuropathology of AD, including neurofibrillary tangles, vascular damage, inflammatory responses and cell death (Selkoe, 1994). A β is generated by the sequential proteolytic processing of the APP gene, resulting in the formation of peptides 40 to 42 amino acids in length. These soluble peptides spontaneously aggregate to form A β oligomers and fibrils that are subsequently deposited within the brain to form diffuse and dense amyloid plaques. The APP gene generates transcripts for coding for the production of A β , the central

component of neuritic plaques that is generated from APP by cleavage by the β - and γ -secretases. The amyloid cascade hypothesis states that $A\beta$ or APP cleavage products which contain $A\beta$ are neurotoxic and lead to tangle formation and cell death. Some research has indicated that $A\beta$ itself is not neurotoxic, but that its presence causes weakening of the neurons, making them more sensitive to excitotoxic damage (Mattson et al., 1992). It is not immediately clear how $A\beta$ causes neuronal loss and tangle formation, however, the peptide is known to disrupt intracellular calcium stability and increase calcium concentrations within the neuron. This could explain how tangles are formed as they are largely composed of hyperphosphorylated tau, which is controlled by the intracellular calcium (Hardy & Higgins, 1992; Mattson, 2004). It is suggested that abnormal deposition or clearance of $A\beta$, identified by CSF $A\beta_{42}$ or PET imaging, could be the earliest marker of AD (see Figure 1.2, Jack et al., 2010).

In people with DS, the amyloid cascade hypothesis is arguably more important than in any other AD population, as the APP gene is located on chromosome 21, and is therefore inherited in triplicate in people with DS. In cases of DS where there is a partial triplication of chromosome 21, one that does not include an additional copy of the APP gene, there are reduced incidences of AD (Korbel et al., 2009; Prasher et al., 1998). When there is presence of an extra copy of the APP gene, it is likely that this leads to a lifelong overproduction of $A\beta$ protein (Hyman, 2002). This has provided much support for the amyloid theory and has been instrumental in determining research efforts (Korczyn, 2008).

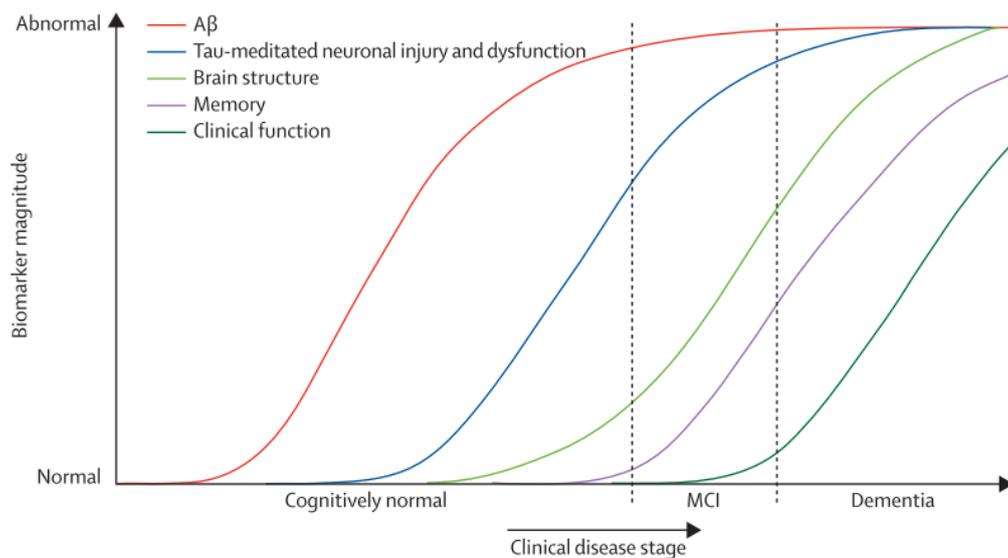


Figure 1.2 A model of the earliest biomarker changes seen in AD. Figure reproduced from Jack et al., 2010, p.122.

AD is a multi-factorial disease with many contributors, and many influencing factors, as shown in Figure 1.3. Evidence to date has shown that although it is necessary, the presence of amyloid alone is not sufficient to result in AD (Kim et al., 2013), therefore other factors must be considered. Nevertheless, the connection between AD and A β is robust, and the amyloid theory will remain a prominent factor in the consideration of the pathophysiology of AD and particularly so in AD in people with DS.

1.3.4.2 *Apolipoprotein E allele inheritance*

The apolipoprotein E (ApoE) gene is influential in the risk of AD, allele type ϵ 4 increases the risk of disease (Hyman, 2002; Patel et al., 2011), whilst inheriting the ϵ 2 allele is considered protective against development of AD (Royston, et al., 1994). In people with DS, the majority of studies have found no discernible differences between the rates of ApoE inherited genes than is found in the typically developing population, particularly noting that the allele ϵ 4 is not more frequently inherited in DS (van Gool, Evenhuis, & van Duijn, 1995; Wisniewski, Morelli, Wegiel, Wisniewski, & Frangione, 1995). However, Sekijima et al., (1998) found higher prevalence of the allele ϵ 4 in patients with DS who developed AD before the age of 50 years (28.6%). This study concluded that the ApoE ϵ 4 is likely to further increase the risk factor of AD in people with DS.

1.3.4.3 *Inflammatory responses*

Neuroinflammation has an important role in AD and accelerates the pathological process (Lott & Head, 2005). Inflammatory responses are evident in many brain diseases besides AD, however, in AD there is a unique association between chronic inflammation and disease, with a strong indication that inflammation serves as an additional cause of AD (Herrup, 2010), rather than being protective against further deterioration. Long-term use of anti-inflammatory drugs have been found to lower the risk of AD (McGeer, Schulzer, & McGeer, 1996). Inflammation is arguably even more important in the DS model, certain genes that are responsible for regulating inflammatory processes are located on chromosome 21 (Wiseman et al., 2015) and impact cognitive impairment and neurodegeneration (Wilcock, 2012). The inflammation argument has been expanded to propose that AD requires three steps; (1) injury that initiates changes distinct from normal ageing, (2) establishment of a chronic inflammatory state and, (3) shift in cellular physiology, a tipping

point which marks the beginning of the degenerative process, leading to synaptic dysfunction and neuronal death (Herrup, 2010).

Specifically microglial mediated inflammation is known to contribute to the progression of AD (Mandrekar-Colucci & Landreth, 2010). Microglial cells form the primary immune system of the brain and are the integral component of maintaining brain homeostasis and protecting the brain from infections. Microglia are present at the sites of A β plaques and increase in number and size in relation to the deposits (Sasaki et al., 2002; Wegiel et al., 2001). Whilst initially microglia exert a neuroprotective role, working to clear soluble A β , research has shown that they are unable to phagocytose A β plaques (Frackowiak et al., 1992). The role of microglia in AD is disputed and some suggest that microglial activation contributes to excessive degeneration. Researchers have suggested that AD represents a failure of neuronal cell cycle control (Arendt, Brückner, Mosch, & Lösche, 2010; Yang, Mufson, & Herrup, 2003), and there is a strong case for the central role in this failure being neuroinflammation (Cameron & Landreth, 2010; Heneka & O'Banion, 2007; Krstic & Knuesel, 2012; McGeer, Schulzer, & McGeer, 1996; Mosher & Wyss-Coray, 2014). As with many mechanisms in AD, it is unclear whether inflammation is a cause, contributor or a by-product in the disorder (Wyss-Coray et al., 2012).

1.3.4.4 *Tau protein hyperphosphorylation and aggregation*

Tau protein are abundant in the neurons of the central nervous system (CNS) and provide structure to assist in the clearance of unwanted and toxic proteins. Excessive or abnormal phosphorylation of tau results in neurofibrillary tangles and allows toxic proteins, including A β , to accumulate inside the neurons and exert toxic effects on the cell, ultimately leading to cell death (Lonskaya, Hebron, Chen, Schachter, & Moussa, 2014). Hyperphosphorylated forms of tau are the main constituent of neurofibrillary tangles. Tau protein is most profusely expressed in the axons in the CNS neurons (Šimić et al., 2016) and there is a strong correlation between the anatomical location of tangles and the greatest cell loss in AD (Herrup, 2010). Studies have shown that AD mouse models carrying human tau mutations develop late-onset neurodegenerative disease, including high levels of neuronal loss (Andorfer et al., 2005; Ramsden et al., 2005). The role of tau in AD is complex, mutations in the tau gene have not been identified in familial forms of AD, but have in other late-onset forms of dementia (Hutton et al., 1998; Poorkaj et al., 1998). Herrup (2010) proposed that

the role of tau in AD occurs in the later stages, and that the phosphorylation of tau is a mechanistic part of programmed cell death. This theory is contradicted by the early appearance of tau tangles in the AD process; consequently, it is likely that tau holds several roles in the progression of AD.

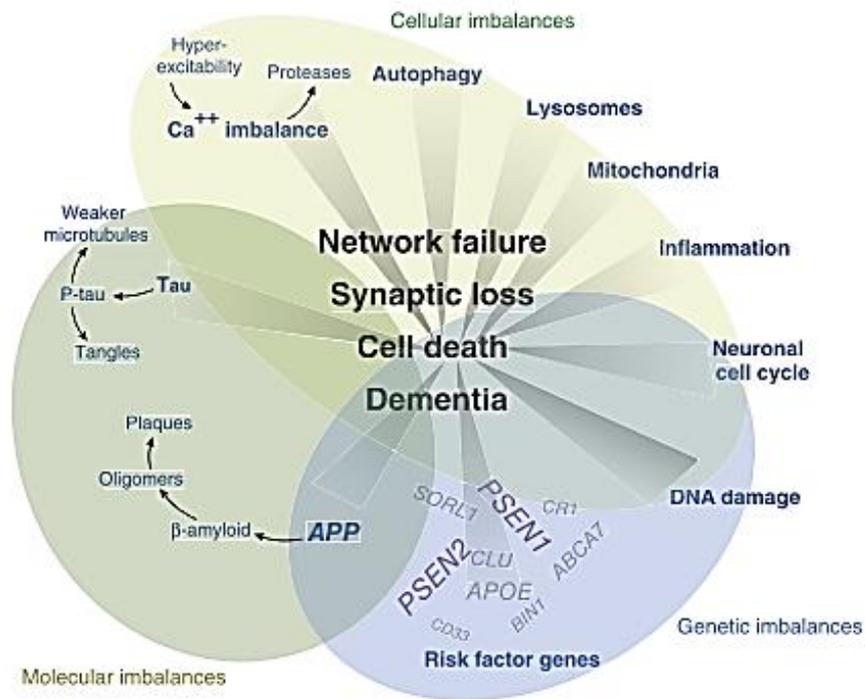


Figure 1.3 Venn diagram showing the degenerative events which ultimately lead to AD. Figure reproduced from Herrup, (2015), page 798.

1.3.5 Therapeutic targets

Biomarkers are defined as being, “characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention” (Atkinson et al., 2001). As with all degenerative diseases, biomarkers are critically important for improving the possibilities of the identification, diagnosis and prevention of AD. Due to the similarities between the progression of AD in people with DS and those without, it is likely that any biomarkers identified in the DS population will also be relevant for the typically developing population. In people with DS, the age of dementia onset is considerably earlier, and clinical presentation is different to that of the typically developing population, however, the neuropathology of the disease is virtually identical, as is the neurodegenerative pathway.

For people with DS, treatments of AD have typically focussed on targeting the amyloid pathway. Mutations in APP that occur close to the beta-site are known triggers for the production of A β , including toxic A β 42 which can lead to early-onset dementia (Selkoe, 2001). β -site APP cleaving enzyme 1 (BACE 1) is up-regulated in people with DS, resulting in increased production of A β (Sun, Tong, Qing, Chen, & Song, 2006). Mutations in the APP gene have been reported in data from individuals of Icelanders, these mutations have been found to protect against AD and cognitive decline in the elderly (Jonsson et al., 2013). Results of this study proposed that reducing the β -cleavage of APP would protect against AD. A recent study using BACE inhibitors in a rat model found a significant reduction in the A β levels seen in the brain and in cerebrospinal fluid (Jian Jeffrey Chen et al., 2015), however this has not yet been trialled in humans. The γ -secretase enzyme is the last stage of APP cleavage before the production of A β (Golde, 2005). Netzer et al., (2010) found that γ -secretase inhibitors in a mouse model of DS successfully lowered A β levels. Interacting proteins with γ -secretase have also been studied, in DS mouse models inhibition of γ -secretase activating protein facilitates A β production without the toxicity effect (Chu, Wisniewski, & Praticò, 2016).

Targeting the inflammation pathway in AD has also been proposed. Several clinical trials using anti-inflammatory drugs have been undertaken and have been shown to prevent A β aggregation in-vitro in human samples (Akiyama et al., 2000; Prati et al., 2013). As a treatment, there have been mixed results, Cuellar et al., (2010) found improved behavioural problems in a DS mouse model, as well as reduced inflammation and reduced A β in the brain, whilst Aisen et al., (2003) found in human patients with AD that there were no changes in cognition. Immunotherapy is also proposed, including several vaccines to protect against synthetic AB42, A β protein and passive immunisation with antibodies (Delrieu, Ousset, Caillaud, & Vellas, 2012). Schenk et al., (1999) has been successful in preventing A β plaque formation and over-expression of APP in an AD mouse model. In humans, the results of this trial showed slower progression rate brain of pathology with the use of A β 42 antibodies, however this was accompanied by the development of sub-acute meningoencephalitis, which resulted in the study being prematurely terminated. Vaccines tested in DS mouse models have shown improvements in cognition, and to be able to prevent neuronal atrophy without visible side effects (Belichenko et al., 2016). Antibodies including solanezumab, ganternerumab, crenezumab and aducanumab are currently undergoing clinical trials. Currently no treatment trials have been conducted in human patients with DS. The

identification of early biomarkers for AD in people with DS is important as such proxy markers for the disease process are necessary if preventative, as opposed to symptomatic, treatments are to be evaluated over an acceptable time scale. Biomarkers could be indicative of the disease mechanisms and can be a proxy outcome measure in clinical trials aiming to prevent or slow the onset of AD.

1.4 The retina in Alzheimer's disease

1.4.1 Retinal development and structure

The retina is derived from the neuroectoderm and retains neuronal, vascular and blood-neural barrier parallels with the brain throughout life. This link to the brain, coupled with the fact that the retina is the only organ of the body that is visible using non-invasive imaging technology, has made it an attractive option for imaging markers and pathology of neurological diseases (Nguyen et al., 2017). As an extension of the brain, it is suggested that AD changes occurring in the brain may also occur in the retina (Parnell, Guo, Abdi, & Cordeiro, 2012).

The retina is part of the CNS and is derived from the neural tube, diverging from the brain during early development (Guo, Duggan, & Cordeiro, 2010; Huseyinoglu et al., 2013; Parnell et al., 2012; Salobar-García et al., 2016). The major development of the eye takes place during the foetal stage, between the third and tenth weeks. The optic vesicles develop on the side of the developing forebrain and extend towards the surface ectoderm. As they grow, the connections to the forebrain become the optic stalks, which eventually form the optic nerves. The developing optic vesicles and stalks have grooves called the choroidal fissure, through which blood vessels supply the optic cup and lens. The choroidal fissure will ultimately fuse, enclosing the vessels in the optic stalk. The optic cup is a double layered structure which splits into two unequal layers (Reese, 2011). The smaller outer layer develops first and melanin granules appear at around 4.5 weeks, becoming the pigment layer. The inner layer is thicker and more complex, ultimately forming the neural retina, cells begin to differentiate into photoreceptors essential for night and colour vision at around six weeks. Above these, towards the inner eye, Müller cells and bipolar neurons are formed, followed by the innermost level which includes the axons of the ganglion cells (Sung & Chuang, 2010). Ganglion cell fibres fill the optic stalk as it becomes the optic nerve. At birth the photoreceptors are still not fully matured, visual acuity will continue to improve for some

time. The retina preserves its connection with the brain through the fibres in the optic nerve. The transparency of the eye means that the retina is the only tissue in the body where neurons can be imaged completely non-invasively.

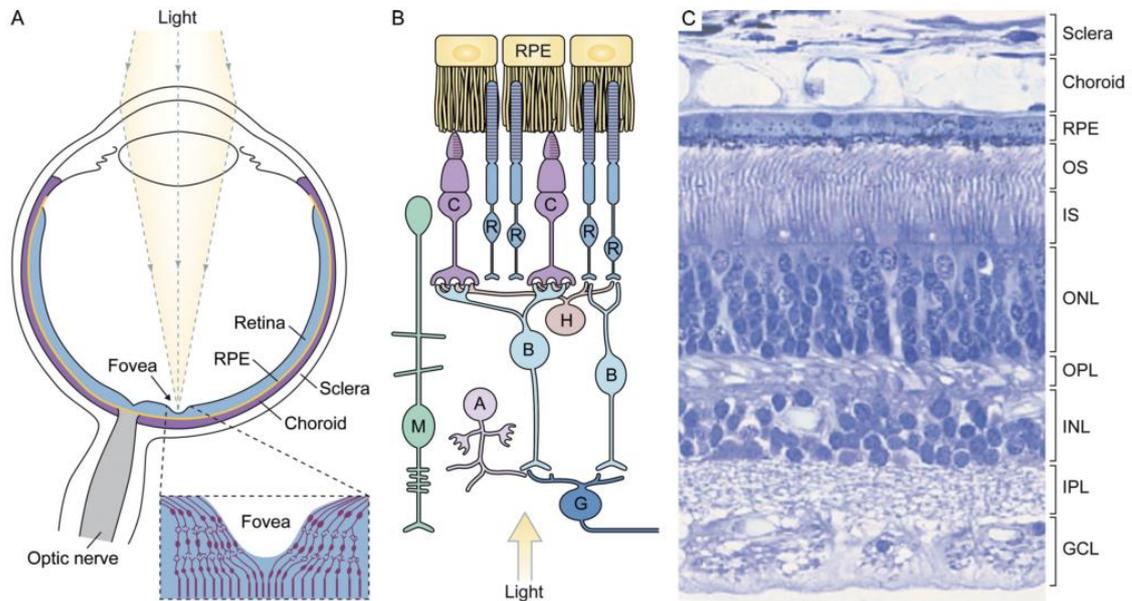


Figure 1.4 Biological arrangement of the eye, cells and retina. (A) Diagram of the eye, (B) diagram of the organisation of retinal cells; Rod, Cone, Bipolar Cell, Horizontal cell, Amacrine cell, Ganglion cell, Müller cell, and, (C) transverse section of the human retina. Figure reproduced from Sung & Chuang, (2010) page 954.

The optic cup has two walls, an inner and outer wall, which form the layers of the retina. The pigmented retinal layer develops first, from the outer wall of the optic cup. The neural layer of the retina is formed from the inner wall of the optic cup. These neural layers are divided into several discrete layers and contain numerous cell types including rods, cones, bipolar and ganglion cells, in addition to the supporting tissue. The retina is divided into different areas with laminated layers composed of different cell types (Figure 1.5). The retinal pigment epithelium (RPE) is a single layer of cuboidal cells closest to the choroid, it has many functions, including light absorption and phagocytosis of photoreceptor membrane segments and is able to communicate with the immune system in order to activate or halt immune system responses (Bok, 1993). The nuclei of the photoreceptors (rods and cones) constitute the outer nuclear layer (ONL), whilst the nuclei of the bipolar, amacrine, horizontal and Müller cells are found in the inner nuclear layer (INL). The nuclei of the

ganglion cells form the ganglion cell layer (GCL) and the nerve fibre layer (NFL) contains the axons of the ganglion cell nuclei. The outer plexiform layer (OPL) comprises the processes and synaptic terminals of photoreceptors, horizontal and bipolar cells. The inner plexiform layer (IPL) contains the processes and terminals of bipolar, amacrine and ganglion cells. The foveal pit is responsible for sharpest central vision but is least sensitive to light. All other space in the retina which is not occupied by neurons and blood vessels is concerned with the processes of the Müller glial cells (Swaroop & Zack, 2002).

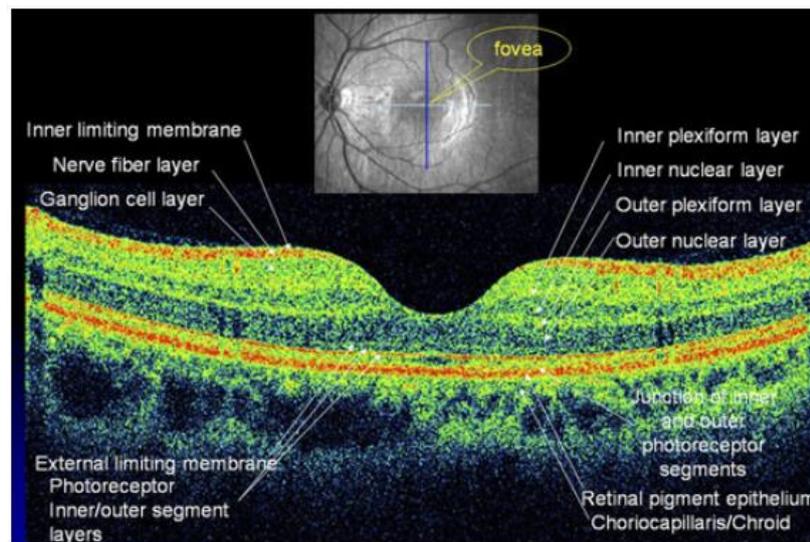


Figure 1.5 Optical coherence tomography (OCT) scan showing a cross-sectional image of the different retinal layers. Figure reproduced from Ikram, Cheung, Wong, & Chen, (2012) page 921.

In people with DS it is assumed that the embryological formation of the eye and the retina is the same as in typically developing foetuses. Despite the increased rates of ocular disorders seen in children and adults with DS, there is no research to indicate that there are any developmental differences in eye development. Features of the eye which have been noted as different in people with DS include; the number of blood vessels which cross the margin of the optic nerve, in many studies the number has been shown to be increased in people with DS (Caputo et al., 1989; Da Cunha & Moreira, 1996; Kim et al., 2002; Williams, McCormick, & Tischler, 1973). This has been referred to as a spoke-like appearance. Williams et al., (1973) found that there was an average increase of 4.2 vessels in the DS group (males and females aged between 10-25 years) compared to an age-matched control group. This increase in blood vessels number has not been shown to impact visual acuity (Da Cunha & Moreira, 1996). In addition to this, thicker retinal layers have been identified

in a DS mouse model (Laguna et al., 2013). Increased retinal thickness was attributed to the extra copy of the DYRKA1A gene which is inherited in triplicate on chromosome 21 in DS. With deletion of this extra copy retinal layers were not dissimilar in thickness from diploid mice. Overall there is no research to suggest that the structure of the retina should be different in people with DS. There are known differences in the development of the DS brain which, considering the connection between the brain and the retina, and that they are formed from the same tissue, may indicate that there could also be differences in retinal formation which are so far unreported.

1.4.2 Age-related retinal diseases

Ageing has a profound impact on almost every aspect of our bodies; the eyes are no exception to this and vision loss and blindness are dramatically increased in older age. Around middle age the lens begins to lose flexibility, reducing our capability to focus on near objects and our sharp visual acuity. For the majority of people this is compensated for by wearing reading glasses or bifocal lenses, but it is seldom fully restored. Vision loss is further worsened if cataracts or other ocular diseases are also present, in these cases, surgery or further intervention, is often required. Muscle weakening becomes a large problem for the eyes as we get older, decreased muscle strength results in drooping eyelids which obscure vision and slow pupil size changes, resulting in increased sensitivity to light (Krinsky-McHale et al., 2012). Most frequently reported ocular diseases in elderly people include; cataracts, age-related macular degeneration (AMD), open-angle glaucoma, detachment of the retina and visual impairment (Klein & Klein, 2013). AMD and glaucoma are particularly relevant in the discussion of AD as in the eyes these diseases can present with some similar pathology to that identified in the eyes of patients with AD. Differentiating between the three diseases will be crucial when evaluating the potential of the retina as a biomarker in AD.

Age-related macular degeneration is a result of damage to the macula region of the retina, risk of AMD can be increased by smoking and genetic factors. It is a painless disease that gradually builds from selective areas of blurred vision, to blindness in the central field. Glaucoma causes damage to the optic nerve and vision loss, this disease causes severe pain in the eye, blurred vision, dilated pupils, redness and nausea. Risk factors include; increased eye pressure, high blood pressure, obesity and family history of the disease. If treated early

it is possible that the disease can be slowed or stopped using medications, laser treatments or surgeries that are aimed at reducing the eye pressure. Glaucoma is the second-leading cause of blindness worldwide.

Glaucoma and AMD, as well as AD, present with depositions of A β , retinal damage, oxidative and metabolic stress and glial activation (Krantic & Torriglia, 2014). Similarities are greater between AD and glaucoma than between AD and AMD (see Figure 1.6). The location of retinal damage and glial activation is important, damage to the optic nerve head (ONH) is particular to glaucoma, whilst retinal ganglion cell loss and nerve fibre loss damage relate to both AD and glaucoma. An increase in intra-ocular pressure is specifically associated with glaucoma (Krantic & Torriglia, 2014). Crucially, cognitive impairments seen in AD are not evident in patients with glaucoma or AMD. In people with DS, who have a genetic predisposition for AD pathology and almost certainty of expressing A β plaques, there is a greater probability that amyloid-related retinal pathologies are part of the AD process, rather than caused by other retinal diseases.

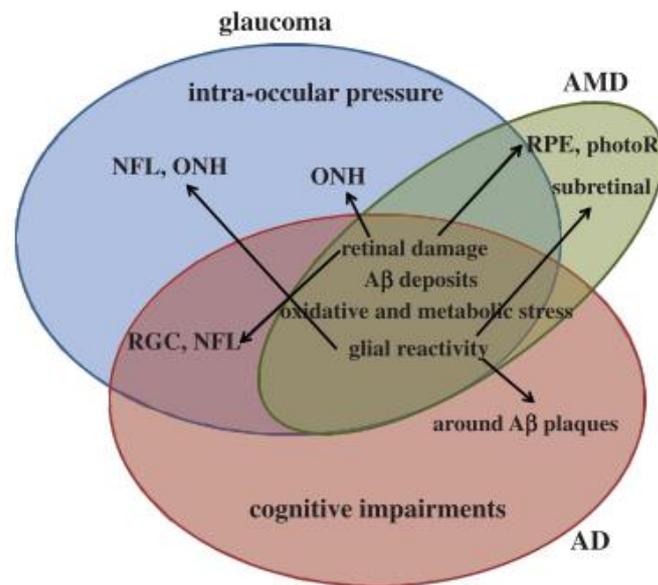


Figure 1.6 Venn diagram showing similarities and differences in the presentation of glaucoma AMD and AD in the retina. Figure reproduced from Krantic & Torriglia (2014), page 241.

1.4.3 Retinal markers in neurological diseases

Many neurological diseases have been found to show visual symptoms and to have the potential for ocular biomarkers. In multiple sclerosis (MS), one of the primary manifestations in the eye is optic neuritis, which affects 70% of MS patients (Balcer, Miller, Reingold, & Cohen, 2015). Optical coherence tomography (OCT) studies in MS have identified reductions in macular thickness and volume (Parisi et al., 1999) and have revealed that there is a RNFL thickness threshold, patients with RNFL thickness under this threshold have a dramatically higher risk of disability (Martinez-Lapiscina et al., 2016). RNFL thinning in the temporal quadrant has been identified as the most useful measure in MS (Graham et al., 2016; Outteryck, Majed, Defoort-Dhellemmes, Vermersch, & Zéphir, 2015), as this region has shown the most sensitivity and specificity for detecting disease progression (Graham et al., 2016) and for distinguishing from other diseases (Outteryck, Majed, Defoort-Dhellemmes, Vermersch, & Zéphir, 2015). In addition to assessments of retinal thickness, clinical assessments of contrast sensitivity and colour vision have been found to be useful in detecting MS patients with optic neuritis. In Parkinson's disease, studies have reported RNFL and macular thinning (Bittersohl et al., 2015; Chorostecki et al., 2015; Garcia-Martin et al., 2012). The majority of studies have reported that, as with MS, the temporal RNFL quadrant is most affected (Pasol, 2011), although thickening of the OPL has been reported compared to healthy controls (Chorostecki et al., 2015). Other measures including contrast sensitivity and visual field tests have been found to successfully differentiate patients with Parkinson's disease from controls.

1.4.4 Retinal changes in Alzheimer's disease

The retina shows evidence of change during the course and progression of AD, which are summarised in this section. Often the first complaints of patients with AD are associated with changes in their vision (Berisha, Feke, Trempe, McMeel, & Schepens, 2007). Visual impairments in people with AD include; reduced visual acuity and visual attention, deficits in perceiving shape from motion, poor object and face recognition, delayed pace of visual processing and reduced visual sensory impairments such as contrast sensitivity or achromatopsia (Koronyo, Salumbides, Black, & Koronyo-Hamaoui, 2012; Parisi, 2003). These visual changes are likely a response to some of the many alterations in the retina which occur during AD.

Retinal ganglion cell loss and optic nerve degeneration in patients with AD was first reported by Hinton, Blanks & Miller, (1986), in a histological research study which showed widespread axonal degeneration of the optic nerve fibres of 80% of patients as well as a reduction in ganglion cells and the thickness of the RNFL. This study also evaluated retinal A β deposits, however these were not found in the AD patients. Blanks, Torigoe, Hinton, & Blanks, (1996) continued this research, finding that the neuronal cell count in the GCL was reduced by 36% in eyes from people with a diagnosis of AD compared to age-matched eyes from people without evidence of AD. It was reported that the largest loss was seen in the superior and inferior quadrants and a 43% reduction of cells in the central fovea was found between AD and control participants (Blanks, Schmidt, et al., 1996). A more thorough report on RNFL reduction in AD can be found in section 1.5.

Presence of amyloid-beta plaques has been identified in the retina both in histopathological studies in animals, where amyloid-beta accumulation has been shown in all retinal layers and in the retinal blood vessel walls (Du et al., 2015; Dutescu et al., 2009; Kam, Lenassi, & Jeffery, 2010; Perez, Lumayag, Kovacs, Mufson, & Xu, 2009; Shimazawa et al., 2008). Deposits were shown to significantly increase with age in the mouse models (Ning, Cui, To, Hsiao Ashe, & Matsubara, 2008). These deposits have also been seen in human patients with AD (Frost et al., 2014; Koronyo-Hamaoui et al., 2011; Koronyo et al., 2017). However other research has suggested that A β does not accumulate in the eye. Michael et al., (2013) and Ho et al., (2012) were not able to show evidence of A β deposits in the lens, retina or other structures of the eye in patients with AD, whilst they were able to detect A β in brain samples using the same techniques, from which the authors concluded that A β either does not deposit in the eye in a manner which is reflective of the brain, or presents in lower levels or different forms. In vivo examinations of the retina have yielded further evidence of amyloid-beta, Hintersteiner et al., (2005) used near-infrared with fluorescence dye, which penetrates the blood-brain barrier and binds to amyloid plaques, to demonstrate significant A β binding in transgenic AD mice brains, which was confirmed by post-mortem analysis. Other studies using curcumin staining in mouse models have shown promising results in both the brain (Garcia-Alloza, Borrelli, Rozkalne, Hyman, & Bacskai, 2007; Yang et al., 2005), and the retina. Koronyo-Hamaoui et al., (2011) provided the first evidence of A β plaques in the retinas of AD transgenic mouse models in vivo. They reported that the in-vivo plaque size detection was very similar to the sizes observed by immunostaining ex-vivo. A β plaques were detected in the retinas and brains of the AD transgenic mice, but not in wild type control

mice. An age-dependent correlation between plaque deposition in the retina and the brain was found which indicated that there was increased accumulation in both alongside disease progression. One of the key findings of this study is that plaques were detectable in the mouse retina at the age of two months, and not in the brain until five months of age, implying that retinal A β could be an early marker for A β in the brain. More recently, a new technology, NeuroVision (NeuroVision Imaging LLC), has been designed which is able to image retinal A β in-vivo. This technology uses curcumin fluorescence to produce micron level high-resolution images of the retinal plaque deposits. Curcumin is a component of turmeric which binds to the A β protein with natural yellow colouring and fluoresces under blue-light excitation. The NeuroVision Retina HD is a fundus camera with a filter specifically matched to the fluorescence characteristics of curcumin. Quantitative analysis of A β plaque number (μm^2) and distribution are derived from the collected images. Emission signals of the plaques are compared to the background signals in the retinal tissue to determine the signal-to-background ratio. NeuroVision calculates the retinal amyloid index in a blinded fashion for each subject. Using this technology A β has been identified in the retinas of patients with AD (Frost et al., 2014; Koronyo-Hamaoui et al., 2011; Koronyo et al., 2017). These deposits mirrored the quantity identified in the brain in histological examinations (Koronyo et al., 2017). Koronyo et al., (2017) found that in twenty patients with a diagnosis of mild cognitive impairment (MCI) between the ages of 66-84 years, A β plaques were imaged and compared to the images of age-matched controls. This study showed higher deposit number and clusters of A β deposits in the periphery of the superior and inferior quadrants and along the blood vessel walls compared to healthy controls. In adults with DS, Rafii et al. (2015) has identified the presence of A β in the retina using the same technology as Koronyo et al (2011). Twelve participants between the ages of 30 and 60 years with DS and without presence of dementia were included in this study. Results showed that there was significant evidence of retinal A β (based on the retinal amyloid imaging scale) in all participants, it was also noted that the distribution of A β in the retina showed a tendency to distribute close to the blood vessels. No correlations were found between age and retinal A β index in this study, the authors attribute this to a small age range (30-60 years), furthermore no correlations were found between A β and cognitive scores. The importance of retinal imaging would be increased if it can be shown that AD-related changes occur in the retina prior to in the brain, as has been suggested by AD-transgenic mouse model research (Koronyo-Hamaoui et al., 2011).

The presence of A β close to the retinal blood vessels may have additional effects on the blood vessels. Other changes seen in the blood vessels include a slower retinal blood flow in patients with AD and systematic changes in parameters such as increased blood pressure (Waldstein et al., 2008) and arterial stiffness (Elias et al., 2009). Increased blood pressure in transgenic mice has been associated with higher concentrations of A β plaques in the brain (Golzan et al., 2017) suggesting a direct relationship between A β and retinal changes. Furthermore, changes in retinal blood vessels have been correlated with declining cognition (Golzan et al., 2017).

Deposits of drusen are frequently seen in the eyes as we get older. Drusen containing A β has been localised to drusen found exclusively in individuals with AMD, whilst drusen in healthy patients does not contain A β (Dentchev, Milam, Lee, Trojanowski, & Dunaief, 2003). In AMD, drusen are one of the earliest visible clinical changes, Peripheral drusen have been found to be significantly associated with AD patients (Aslam et al., 2014; Lengyel et al., 2015; Ritchie et al., 2011). These studies investigated the similarities in retinal features between AMD and AD and found that distribution of the A β containing drusen was specifically located in the peripheral retina in people with AD. In a longitudinal study, Aslam et al., (2014), analysed drusen and cognitive ability in healthy controls and patients with AD over the course of two years, findings showed increased drusen in all participants, however this progression was significantly more prevalent in the AD patients.

In people with AD there are noticeable changes in the optic disc and the optic nerve. In the optic disc there are changes in the colour of the disc pallor, becoming a pale yellow in colour rather than the typical pink tones. Optic atrophy and disc cupping is also seen in patients with AD (Kiyosawa, Bosley & Chawluk, 1989; Sadun, Borchert, DeVita, Hinton, & Bassi, 1987). Optic disc cupping is another feature which is one of the primary characteristics of another retinal disease, glaucoma, and is also evident in Parkinson's disease (Ikram et al., 2012). In the optic nerve, there is a decline of nerve axons, predominantly affecting the large-diameter axons (Syed, Armstrong, & Smith, 2005; Tsai et al., 2014).

Finally, there is evidence of A β deposits in the lens of the eye in patients with AD. The levels of A β identified in the lens is comparable to those seen in the brain (Goldstein et al., 2003). Several types of cataracts are associated with an increase in insoluble proteins (Frederikse et al., 1996) and these are also characteristic of people with AD. In a DS study, a link has been established between the risk of cataract formation and AD, showing a link

between lens pathology based on the distribution and accumulation of A β in this area (Moncaster et al., 2010). However, in typically developing AD lenses of donor with AD have not consistently found evidence of A β , Michael et al., (2013) was unable to locate A β whilst Goldstein et al., (2003) and Moncaster et al., (2010) provided strong evidence of A β in AD lens cataracts.

1.4.5 Retinal structure in Down's syndrome

Investigations of retinal structure in people with DS using OCT are very limited, a literature search of PubMed and Scopus databases revealed a total of just five studies (Table 1.1). Of these studies, two were case studies focussing on individuals with particular ocular abnormalities, which cannot be applied more generally (Altun, Altun, Kurna, Olcaysu, & Aki, 2014 and Aziz, Ruggeri, & Berrocal, 2011), furthermore these studies did not report on retinal thickness.

Two studies, O'Brien et al., (2015) and Weiss, Kelly, & Phillips, (2016) conducted cross-sectional OCT examinations in children with DS and compared findings to typically developing children. Retinal thickness was assessed in both studies. O'Brien et al., (2015) found thicker fovea and macular in DS children (17 children aged between six and 16 years). The foveal pit was noticeably shallower in images of the retina and significantly thicker in the DS group ($281\pm 17\mu\text{m}$ compared to $246\pm 21\mu\text{m}$ in the control group). Macular quadrants were significantly thicker in all except the temporal quadrant. Inner retinal layers were more similar in thickness between groups, whereas the outer layers again showed increased thickness in children with DS. One of the limitations reported by the authors was that due to low quality of the images they were unable to provide full segmentation of individual layers. It was proposed that thicker macular could be a primary pathology related to the disorder. Weiss et al., (2016) conducted a smaller OCT study in seven children with DS (age seven to 15 years). This study reported that all retinal layers appeared normal in all eyes, but that the fovea was increased in thickness specific to the ONL in the DS group.

The final study examined five eyes of three adult participants with DS (29.2 ± 16.6 years) using OCT (Laguna et al., 2013). The OCT component of this study was secondary to investigations of retinal thickness in a mouse model of DS (Ts65Dn) and optical biopsies of DS retinas. From the OCT examinations, they reported that retinal structure was not

dissimilar to age-matched controls. From the biopsies of 16 retinas, it was reported that the fovea and inner macular (all quadrants) were thicker than control retinas, although a significant difference was only seen in the fovea region. The DS mouse model component of the study also showed evidence of thicker retinal structure when compared to wild type; particularly the inner retinal layers, whilst the outer retinal layers were more similar in thickness (Laguna et al., 2013). One of the genes in triplicate in the Ts65Dn mice is *Dyrk1a*, a separate mouse model, mBACtgDyrk1a, containing specific trisomy of this gene was also used in this study, and exhibited increased retinal thickness similar to that of the Ts65Dn mouse. However, removal of the third copy of this gene resulted in normal retinal thickness. This study suggests that the inhibitory effect of *Dyrk1a* on apoptosis during development may be responsible for the increased thickness of the retina.

Due to the limited research in DS it is difficult to reach a conclusion on retinal structure; it appears that there is a trend towards increased thickness in some retinal areas, although this deduction is predominantly based on findings in children with DS. In adults in the typically developing population there is strong evidence of age-related thinning of the retina, and of significant thinning in AD patients (discussed in detail in section 1.4). Adults with DS present with accelerated ageing and have high prevalence of AD, therefore, may be more likely to show increased retinal thinning. To date there has been no large-scale research on retinal thickness in adults with DS. This study is the first to build on the findings of OCT research conducted in typically developing AD into adults with DS.

Author	Sample (DS)	Main findings
Altun, Altun, Kurna, Olcaysu, & Aki. (2014)	1 patient, aged 15 years	Reduced retinal nerve fibre layer (RNFL) thickness in the right eye. Case study of a patient with unilateral morning glory optic disc anomaly.
Aziz, Ruggeri, & Berrocal (2011)	1 patient, aged 3 years	Absence of the retinal pigment epithelium and atrophy of the neurosensory retina consistent with diagnosis. Case study of a patient with bilateral macular coloboma.
Laguna et al. (2013)	3 patients, mean age 29.2 ± 16.6 years	Thicker neural retina than controls, significantly thicker only in the central fovea region.
O'Brien et al. (2015)	17 children with DS aged 6-16 years	Thicker central subfield thickness and inner and outer layers of the macula in the DS group.
Weiss, Kelly, & Phillips (2016)	7 children with DS aged 7 – 14 years	Retinal layers appeared normal in all eyes, the fovea showed thickening in the outer nuclear layer and central fovea thickness was larger than in non-DS children.

Table 1.1 OCT examinations conducted in DS populations

1.5 A systematic review of retinal nerve fibre layer thinning in patients with Alzheimer's disease.

1.5.1 Literature search

A systematic literature review was undertaken to explore the published research that has used OCT technology to investigate RNFL thickness in patients with AD. The search terms and criteria for the inclusion pathway are illustrated in Figure 1.7. Scopus and PubMed databases were searched to identify published articles in patients with AD or MCI. Meta-analyses were not included for this literature review although references were screened for overlooked literature. Non-human studies and reviews were excluded, as were articles where the primary aim was to compare or develop OCT methodologies, and those that did not compare retinal measures between at least one patient group and a control group. Screening of the article title and abstracts was conducted as a primary eligibility check before thorough investigations of the articles. A total of 36 original research articles were retained for examination. These were further sub-divided into those involving patients with AD and controls, and those which also included an MCI patient group. The full list of retained articles can be found in Table 1.2.

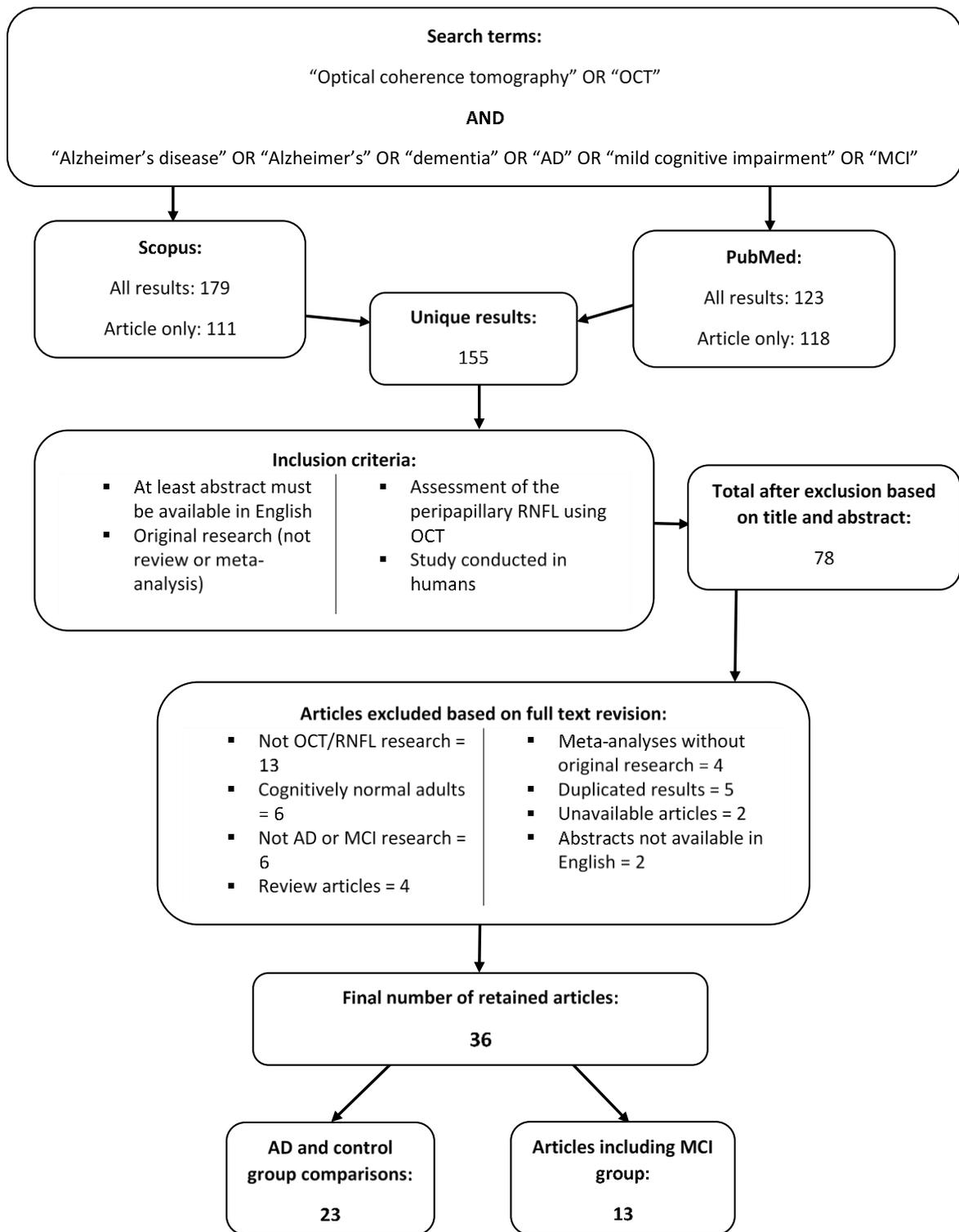


Figure 1.7 Article identification process for the systematic literature review – conducted 20th May 2017.

Author	Sample	Cognitive testing	Key findings
<i>RNFL thickness comparisons between AD and age-matched control groups</i>			
Bambo et al., (2015)	56 patients with AD 56 age-matched controls	Mini mental state examination (MMSE Folstein, Folstein, & McHugh, 1975)	RNFL average and all quadrants except the temporal were significantly thinner in the AD group.
Berisha, Feke, Trempe, McMeel, & Schepens, (2007)	9 patients with AD 8 age-matched controls	MMSE	Only the superior quadrant was significantly thinner in the AD group.
Cunha et al., (2016)	24 patients with AD 24 age-matched controls	MMSE	Superior and inferior quadrant RNFL thickness significantly thinner in AD group.
Eraslan et al., (2015)	20 patients with AD 20 age-matched controls	MMSE	Significant reduction in the RNFL average and superior-nasal thickness of the AD group when compared to the controls.
Garcia-Martin et al., (2016)	150 patients with AD 75 age-matched controls	MMSE	AD patients had significantly reduced thickness in the RNFL average, temporal superior, temporal inferior, temporal compared to controls.
Garcia-Martin et al., (2014)	20 patients with mild AD 28 age-matched controls	MMSE	RNFL thickness did not differ between groups.
Gharbiya et al., (2014)	21 patients with AD 21 age-matched controls	MMSE	RNFL thickness in all quadrants was not significantly different between groups.
Gunes et al., (2015)	40 patients with AD 40 age-matched controls	MMSE	RNFL average and all quadrants significantly thinner in the AD group when compared to controls.

Author	Sample	Cognitive testing	Key findings
Iseri et al., (2006)	14 patients with AD 15 age-matched controls	MMSE	Average RNFL and all quadrants exempting the temporal were significantly thinner in the AD group.
Kang & Kim, (2013)	8 patients with AD 8 age-matched controls	n/a	RNFL average, nasal and inferior were significantly thinner in the AD group.
Kirbas et al., (2013)	40 patients with AD 40 age-matched controls	MMSE	RNFL average and the superior quadrant significantly thinner in AD group.
Kromer et al., (2014)	22 patients with AD 22 age-matched controls	MMSE	RNFL thickness in the nasal superior sector significantly thinner in the AD group.
Larrosa et al., (2014)	151 patients with AD 61 age-matched controls	MMSE	RNFL average, nasal, nasal-inferior, temporal-inferior and temporal superior were significantly thinner in the AD group.
Lu et al., (2010)	22 patients with AD 22 age-matched controls	n/a	RNFL average, superior and inferior quadrants significantly thinner in the AD group
(Marziani et al., 2013)	21 patients with AD 21 age-matched controls	MMSE	All measures of RNFL were significantly reduced in the AD group.
Moreno-Ramos, Benito-León, Villarejo, & Bermejo-Pareja, (2013)	10 patients with AD 10 age-matched controls	MMSE	Significant RNFL thickness decrease in AD group compared to control group.

Author	Sample	Cognitive testing	Key findings
La Morgia et al., (2015)	21 patients with AD 74 age-matched controls	MMSE	Average and superior RNFL measures significantly thinner in the AD group.
Trebbastoni et al., (2016)	36 patients with AD 36 age-matched controls	MMSE	RNFL average and superior and inferior quadrants showed significant reduction in thickness compared to controls over a period of 12 months, inferior quadrant decline was most prominent and correlated with cognitive decline.
Parisi et al., (2001)	17 patients with AD 14 age-matched controls	n/a	RNFL average and all quadrant measures were significantly thinner in the AD group.
Polo et al., (2014)	75 patients with AD 75 age-matched controls	MMSE	Average RNFL and superior, inferior, inferior-nasal and inferior-temporal sectors thinner in AD group.
Polo et al., (2017)	24 patients with AD 24 age-matched controls	MMSE	RNFL average and superior quadrant measures significantly thinner in the AD group.
Salobar-garcia et al., (2015)	23 patients with AD 28 age-matched controls	MMSE	RNFL average and all quadrants significantly thinner in the AD group.
Moschos, Markopoulos, Chatziralli, Rouvas, & M Moschos, (2012)	30 patients with AD 30 age-matched controls	n/a	Mean foveal thickness and superior and inferior quadrants significantly thinner in the AD group.

Author	Sample	Cognitive testing	Key findings
<i>MCI group RNFL thickness comparisons to age-matched control groups and AD groups</i>			
Ascaso et al., (2014)	18 patients with AD 21 patients with MCI 41 age-matched TD controls	MMSE	RNFL was thinner in MCI patients compared to controls, and again thinner compared to the AD patients.
Cheung et al., (2015)	100 patients with AD 41 patients with MCI 123 age-matched controls	n/a	There was no difference in the RNFL between MCI and AD measures. AD and MCI groups differed significantly from the controls in RNFL average and quadrant measures.
Ferrari et al., (2017)	39 patients with AD 27 patients with MCI 17 patients with dementia of the frontal type (DFT) 49 age-matched controls	MMSE	All groups showed significant reduction of RNFL when compared to controls. There was no significant difference between the MCI and AD groups however it was noted that the MCI group had thinner RNFL than those identified as mild AD.
Gao, Liu, Li, Bai, & Liu, (2015)	25 patients with AD 26 patients with MCI 21 age-matched controls	MMSE	AD and MCI groups showed thinner RNFL measures than the control group for the average, and superior quadrant. Furthermore average RNFL, and the inferior quadrant were significantly thinner in the AD group compared to the MCI group. AD compared to controls showed significantly thinner results for the average and the superior, inferior and temporal quadrants.

Author	Sample	Cognitive testing	Key findings
Kesler et al., (2011)	30 patients with AD 24 patients with MCI 24 age-matched controls	MMSE	MCI and AD groups both showed significantly thinner RNFL than the control group for the average, superior and inferior quadrants. There were no statistical differences between the AD and MCI groups.
Knoll et al., (2016)	20 patients with MCI 20 age-matched controls	Test battery of the Uniform data set (Estevez-Gonzalez, Kulisevsky, Boltes, Otermin, & Garcia-Sanchez, 2003)	No significant differences found for any RNFL measures between groups.
Liu et al., (2015)	19 patients with severe AD 24 patients with moderate AD 24 patients with mild AD 26 patients with MCI 39 age-matched controls	MMSE	MCI and mild AD groups were significantly thinner than the control group in the superior and average RNFL measures, for these regions moderate and severe AD also significantly thinner than MCI. Severe AD also showed significantly thinner inferior quadrant than both control and MCI groups.
Oktem et al., (2015)	35 patients with AD 35 patients with MCI 35 age-matched controls	MMSE	Significant thinning with relationship to age was detected. Both MCI and AD groups had significantly thinner RNFL average compared to controls, there was no significant difference between MCI and AD RNFL measures.

Author	Sample	Cognitive testing	Key findings
Paquet et al., (2007)	12 patients with severe AD 14 patients with mild AD 23 patients with MCI 15 age-matched controls	MMSE	All patient groups showed significantly thinner average RNFL measures when compared to the control group. Mild AD group showed non-significantly thicker RNFL average than MCI group.
Pillai et al., (2016)	21 patients with AD 20 patients with MCI 34 age-matched controls	Montreal Cognitive Assessment (MOCA; (Nasreddine et al., 2005))	No statistical significant differences were reported in any RNFL measures between any groups.
Senthilkumar & Prabna, (2016)	14 patients with AD (glaucoma) 12 age-matched controls	n/a	RNFL average and all quadrants significantly thinner in the AD group. <i>Note that this article does not distinguish how patients with AD were identified and does not make a distinction between AD and glaucoma.</i>
Shen et al., (2014)	23 patients with MCI 52 age-matched controls	MMSE-Chinese	No significant difference between the RNFL measures of the groups. MCI group showed an inverse association between cognitive function and the inferior quadrant. Patients with a thicker inferior quadrant performed worse in cognitive tests.
Zhu, Ren, Wang, & Zhang, (2014)	10 patients with AD 47 patients with MCI 167 age-matched controls	MMSE	RNFL average temporal and superior quadrants were significantly thinner in MCI and AD groups compared to controls, only the temporal quadrant was significantly thinner in AD group compared to MCI groups.

Table 1.2 Key information of studies included in the literature review.

1.5.2 Summary and review of identified articles

This literature review has evaluated the differences in RNFL thickness between patients with AD, MCI and control groups. From the studies identified, further investigations were undertaken into the associations between retinal thicknesses and cognitive test scores and into supplementary areas of the retina that may have been investigated, such as the macular and retinal layers.

1.5.2.1 *Thinner RNFL in patients with Alzheimer's disease*

Findings from the literature review indicate that there is a significant difference between the RNFL thickness of AD patients and age-matched control groups. All but three of the studies reported thinner RNFL in the AD patients (Bambo et al., 2015; Eraslan et al., 2015; Garcia-Martin et al., 2016; Gunes et al., 2015; Iseri et al., 2006; Kang & Kim, 2013; Kirbas et al., 2013; Larrosa et al., 2014; Lu et al., 2010; Marziani et al., 2013; Moreno-Ramos et al., 2013; La Morgia et al., 2015; Parisi et al., 2001; Polo et al., 2017; Salobrar-Garcia et al., 2015). Those which did not see thinner RNFL in AD patients found no differences between groups (Garcia-Martin et al., 2014; Gharbiya et al., 2014; Pillai et al., 2016), although one of these studies (Garcia-Martin et al., 2014) reported significantly thinner RNFL in AD patients in a later study with a larger sample (Garcia-Martin et al., 2016). The number of studies that did not show significant results is consistent with expectations based on hypothesised power of 80% for these studies. Furthermore, lack of significant findings in these three studies is not likely to be due to sample size, as studies with smaller sample sizes such as Moreno-Ramos et al., (2013) and Kang & Kim, (2013) with eight and ten participants per group respectively, found significant differences in the average RNFL thickness between groups.

Within the RNFL there are four quadrants that are frequently measured. Of the studies evaluated in this review, three did not provide quadrant analysis (Moreno-Ramos et al., 2013; Oktem et al., 2015; Paquet et al., 2007). From those that did, quadrant analyses almost always showed that all of the quadrants were significantly reduced in thickness in patients with AD (Gharbiya et al., 2014; Gunes et al., 2015; Parisi et al., 2001; Salobrar-Garcia et al., 2015; Senthilkumar & Prabna, 2016). The superior quadrant was most likely to show evidence of thinning in the AD group (Berisha et al., 2007; Eraslan et al., 2015; Kirbas et al., 2013; Liu et al., 2015; La Morgia et al., 2015; Polo et al., 2017) and the inferior quadrant the second most likely, however, thinning in this region was often accompanied by

significant thinning in the superior quadrant (Cunha et al., 2016; Kesler et al., 2011; Liu et al., 2015; Lu et al., 2010; Moschos et al., 2012). The majority of research indicates that the superior quadrant may be the most, and earliest, area of the retina implicated in AD. Although both the inferior and nasal quadrants were reported as thinner in patients AD, no studies found thinning of either quadrant without there also being significant thinning of the superior quadrant (Kang & Kim, 2013; Kromer et al., 2014; Larrosa et al., 2014). Liu et al., (2015) found that the nasal quadrant was reduced in thickness only in severe AD, suggesting that reductions in the nasal quadrant are later in the disease process. The temporal quadrant was found to be significantly thinner in just one study (Garcia-Martin et al., 2016) and in other studies was identified as the only quadrant not to show evidence of thinning (Bambo et al., 2015; Iseri et al., 2006). This suggests that the temporal quadrant is more resilient to fibre loss than other quadrants. Resilience in the temporal quadrant is unexpected as Parkinson's disease and MS have shown specific thinning in this area (Graham et al., 2016; Pasol, 2011). Identifiable differences such as these in the retinal presentation of neurological diseases are imperative if retinal measures are to be used as early markers for the screening of AD. Ultimately, this research suggests that in patients with AD, RNFL thickness is generally reduced, and the superior quadrant shows the most evidence of thinning in comparison to age-matched control groups. Additional thinning, particularly in the inferior quadrant, follows after thinning of the superior quadrant.

1.5.2.2 *RNFL changes in MCI*

Thirteen of the studies identified in the literature search included a MCI patient group. Comparing differences between retinal thicknesses of the MCI group to controls, and also between MCI and AD groups is important to establish the earliest changes in the retina in the AD progression. The majority of studies in patients with MCI found a significant reduction in the average RNFL thickness compared to control groups (Ascaso et al., 2014; Cheung et al., 2015; Ferrari et al., 2017; Gao et al., 2015; Kesler et al., 2011; Liu et al., 2015; Oktem et al., 2015; Paquet et al., 2007; Zhu et al., 2014). Evidence of significant differences in retinal thickness between the AD and MCI groups was not as robust, with equal numbers of studies finding no significant differences between groups (Cheung et al., 2015; Knoll et al., 2016; Oktem et al., 2015; Pillai et al., 2016), and significantly thinner RNFL in the AD group compared to the MCI group (Ascaso et al., 2014; Gao et al., 2015; Liu et al., 2015; Zhu et al., 2014). Two studies identified further subgroups within the MCI/AD

classifications. Paquet et al., (2007) subcategorised the AD group based on scores from the mini-mental state examination (MMSE; Folstein et al., 1975), which resulted in a third group in between AD and MCI termed “mild dementia”. Findings showed no differences between AD and mild AD patient groups, but a significant difference between AD and MCI groups was found. These results suggest that the gradual changes in RNFL thickness are too small to be further sub-divided.

Quadrant analysis for MCI was assessed in all but three of the papers identified (Ferrari et al., 2017; Oktem et al., 2015; Paquet et al., 2007). Ascaso et al., (2014) and Cheung et al., (2015) found that all quadrants were significantly reduced in thickness in the MCI patients compared to the control group, however, no differences were found between the MCI and AD groups. Reduction of thickness was specifically noted in the superior quadrant (Gao et al., 2015; Liu et al., 2015). Likewise, Kesler, Vakhapova, Korczyn, Naftaliev, & Neudorfer, (2011) found that the inferior and superior quadrants were both significantly reduced in the MCI group compared to the control group. Zhu, Ren, Wang, & Zhang, (2014) reported a reduction in the average, temporal and superior quadrant thickness in both MCI and AD patients. Results from studies including an MCI patient group suggest that the pattern of change is very similar to that seen in the AD group, and reinforces the theory that degeneration in the superior quadrant is the most profound and likely to be the first site to show signs of change, and that retinal changes can be seen in the early stages of AD.

1.5.2.3 *Macular changes in AD*

As well as the peripapillary RNFL, several of the studies identified also investigated macular thickness. Decreased macular thickness was found in patients with AD when compared to controls (Cunha et al., 2016; Giménez, Dudekova, Gómez, & Lajara, 2016; Moschos et al., 2012; Polo et al., 2017), as was in MCI patients in compared to controls (Cunha et al., 2016; Liu, Ong, Hilal, Miin, & Wong, 2016; Moschos et al., 2012; Polo et al., 2017). Several studies investigated macular quadrants, and all but one (outer macular inferior quadrant) were reported to be thinner in patient groups (Cunha et al., 2016; Polo et al., 2017). Moschos, Markopoulos, Chatziralli, Rouvas, & Moschos, (2012) reported significantly thinner fovea in AD patients. Controversially, Ascaso et al., (2014) found that although AD patients showed significantly thinner macular than controls, the MCI group had significantly thicker macular than both the AD and control groups. Thicker macular persisted in the MCI group

in both the fovea and in the the average of the inner macular but not the outer macular. Conclusions made from this research were that this finding could be a result of retinal cell activation and swelling. Reichenbach et al., (2007) has reported the activation of perifoveal Müller glial cells, with consequent swelling in the early stages of retinal degeneration. Müller cells are closely linked with retinal neuronal cells, forming much of the non-neuronal retinal space and are susceptible to inflammation (Bringmann & Wiedemann, 2011). Neuronal swelling is a pathological hallmark of several neurodegenerative diseases, including AD (Fujino, Delucia, Davies, & Dickson, 2004) and may result in retinal thickening in the early stages of AD, which is later followed by thinning as inflammation induces programmed cell death processes such as apoptosis.

1.5.2.4 *Retinal thickness and cognitive change*

The relationship between cognitive ability and retinal thickness was investigated in the majority of the studies identified, typically the MMSE (Folstein et al., 1975), was used to determine cognitive status. The MMSE is one of the most widely used tests in the screening of dementia in the typically developing population. Studies mostly reported that there was no relationship between cognitive score and retinal thickness (Eraslan et al., 2015; Ferrari et al., 2017; Gao et al., 2015; Gharbiya et al., 2014; Gunes et al., 2015; Iseri et al., 2006; Kesler et al., 2011; Kirbas et al., 2013; Kromer et al., 2014; La Morgia et al., 2015; Paquet et al., 2007; Polo et al., 2017). Of the few studies that did identify a relationship, lower MMSE scores were invariably correlated with thinner retina (Ascaso et al., 2014; Cunha et al., 2017; Garcia-Martin et al., 2014; Moreno-Ramos et al., 2013; Oktem et al., 2015; Trebbastoni et al., 2016). Within these studies there was extreme variability in the extent of the association found. Moreno-Ramos et al., (2013) reported the strongest significant correlation ($r = .963$, $p < .05$), whilst others reported lower significant correlations ($r = .33$, Cunha et al., 2017; and $r = -.34$, Garcia-Martin et al., 2014). Trebbastoni et al., (2016) conducted a longitudinal study in which the rates of MMSE decline were evaluated after one year, this study was able to associate cognitive decline with greater fibre loss in the inferior quadrant. The inferior quadrant was also identified as having the strongest correlation with MMSE scores ($r = .37$), followed by the superior quadrant ($r = .22$) whilst the temporal and nasal quadrants were not significantly correlated (Cunha et al., 2017). Conversely, Ascaso et al., (2014) found a significant positive relationship between MMSE score and the thickness of the nasal and superior quadrants, as well as global RNFL, but no relationship with the inferior quadrant.

A small number of the articles also included MMSE correlations with the macular region. Cunha et al., (2016) found a stronger correlation with MMSE and retinal thickness in the macular ($r = .49$) than in the RNFL. Iseri et al., (2006) and Ferrari et al., (2017) did not find a relationship between RNFL thickness and MMSE scores, but both studies reported a positive correlation between the thickness of the GCL-IPL macular layers and MMSE scores. Garcia-Martin et al., (2014) identified a positive relationship with the GCL. However, Ascaso et al., (2014) found opposite results, reporting no relationship between MMSE scores and macular region thickness or volume in MCI or AD patients.

In studies where cognitive tests other than the MMSE were applied to the patient groups (Knoll et al., 2016; Shen et al., 2014) an inverse relationship between cognitive tests and RNFL thickness was identified, suggesting that thicker retina was associated with lower cognitive ability. Both studies highlighted the inferior quadrant as the region showing the strongest correlations. Shen et al., (2014) found that thicker inferior quadrant was worse correlated with poorer overall cognitive function and specifically with poorer episodic memory. Knoll et al., (2016) found that in tests of delayed story recall and word-list learning those with poorer scores also displayed thicker retina. These findings suggest that the MMSE is not comprehensive enough to identify all retinal and cognitive relationships. The MMSE is a short, 10-item scale that is easy to deliver in people with cognitive problems thereby increasing its appeal. The issue with applying results from these studies which have used less standardised materials is that there are no replications of the findings.

1.5.2.5 *Conclusions*

Conclusions from this literature review indicate that there is an association between retinal thickness and AD. The majority of studies assessed in this literature review have found significantly thinner RNFL and macular regions in patients with AD and, often, in patients with MCI, suggesting that retinal changes occur at an early stage of the disease. The superior quadrant is highlighted as the quadrant of the RNFL showing the most thinning in patients with AD. Concerning for the use of the retina as a biomarker for AD is that there appeared to be little evidence of correlation between retinal thickness and cognitive change. The inferior quadrant showed the most association with cognitive scores and decline. However, conflicting results in the literature have seen both a thinner inferior quadrant associated with lower MMSE scores, and a thicker inferior quadrant associated with decline in episodic

memory, story recall and word list recall. The confusion surrounding this topic indicates that more research is needed in order to fully understand the involvement of the RNFL in AD.

1.6 Aims and hypotheses

The primary aim of this thesis is to evaluate the potential of studying retinal thickness in adults with DS as a potential marker of AD related change. To achieve this, retinal thickness was examined using OCT technology and dementia status was investigated using informant interviews, neuropsychological tests and, in collaboration with another study in the same DS cohort, amyloid deposition in the brain. Natural ageing in the retina will also be investigated in the DS group in relation to typically developing control groups.

Overall hypotheses:

- People with DS will have thinner retina than age-matched typically developing controls and retinal thinning will be more pronounced with age in the DS group.
- Retinal thickness will correlate with cognitive performance.
- Cortical thickness will positively correlate with retinal thickness.
- Individuals with DS with a diagnosis of dementia, and those with “positive brain A β binding” will show thinner retinal measures than those without.

Chapter Two:

Methods and materials

2.1 Chapter summary

The methodologies used in data collection and analysis are described in this chapter. All results chapters (chapters three, four and five) share data on retinal image acquisition and DS participant recruitment. Results chapters consider; investigation of retinal thickness in adults with DS in comparison to typically developing controls, and in relation to ageing, cognition and dementia in DS. Retinal and cortical thickness associations and retinal thickness in the presence and absence of fibrillar amyloid-beta ($A\beta$) binding in the brain are also explored.

2.2 Collaborations

This study has been conducted in the Cambridge Intellectual and Developmental Disabilities Research Group (CIDDRG) as part of the Defeat Dementia in Down's syndrome (DiDS) studies at the University of Cambridge. DS participant recruitment was conducted in collaboration with an electroencephalography (EEG) study conducted by Dr Jennings. Neuropsychological tests and dementia interview data were jointly collected and used in both studies. Data from a previous neuroimaging study, DiDS, was used in the analysis of Chapter Five. This neuroimaging study investigated brain structure and $A\beta$ in adults with DS over the age of 30 years using MRI and PET imaging and was conducted by Dr Wilson and Dr Annus.

Ophthalmic support for this study was provided by researchers at the Imperial College Ophthalmic Research Group (ICORG), Western Eye Hospital, London. Professor Cordeiro (Professor of Retinal Neurodegeneration and Glaucoma Studies) was an advisor on this project, and Dr Normando (Ophthalmologist and Glaucoma Specialist) conducted the OCT scans and provided medical expertise and analysis guidance.

2.3 Approvals from Regulatory Authorities

Ethical approval for this study was granted by the National Research Ethics Committee East of England – Cambridge Central, on November 3rd 2014 (see appendix A). Research and development approvals were granted by Cambridge University Hospital NHS Foundation Trust and Cambridge and Peterborough Foundation Trust. This project was conducted within the conventions of the Declaration of Helsinki (2013).

2.4 Study design

This cross-sectional study aimed to investigate retinal thickness in people with DS in relation to age, cognition, and dementia status. Retinal thickness was examined in all participants using OCT; scans investigated the thickness of the peripapillary retinal nerve fibre layer (RNFL), and the macular and posterior pole regions including the segmented retinal layers of the posterior pole. Neuropsychological tests were used to measure cognitive functioning in areas known to decline with dementia, an IQ test and an informant interview for dementia status were conducted in the DS group, for control participants a dementia screening assessment was completed. Finally, in a sub-set of DS participants with previous neuroimaging data, cortical and retinal thickness relationships were assessed in the presence and absence of A β binding in the brain, as an indicator of developing dementia.

2.5 Study population

2.5.1 Participant identification

For this study three groups of participants were recruited; people with DS over the age of 18 years, an age-matched typically developing control group and a typically developing group older than the DS group (57 years and over).

Participants with DS were recruited nationally and at least one home visit was made to each participant to explain the study. Identification of people with DS was through one of three avenues. Firstly, participants who had previous involvement in another of the DiDS research studies were invited to take part in this study. All these participants had previously signed a consent form agreeing to be contacted about future studies. Secondly, recruitment through the Down's syndrome association (DSA), the DSA have been very supportive of all of the

DiDS studies for over five years, forwarding materials and identifying participants. Recently, the DSA have supported all of our public engagement events. Public engagement events were the final recruitment strategy utilised in this study. In order to raise awareness about our research, three open day events have been held. On world Down's syndrome day (21st March) 2015 and 2016 an open day was held in Cambridge and an additional public event was held at the DSA headquarters in July 2016. At these events, members of the public, people with DS and their families were invited to meet the researchers and talk about current research. Posters were presented by the researchers explaining what the studies involved and current findings and people were able to ask questions and discuss any concerns they had about the research project. The event held in Cambridge in 2016 also produced a short video, where participants were asked about their involvement in the research and how they felt about taking part in research. This video can be viewed at <https://youtube/pB7iqWUXQIM>. These events also included crafts, activities and refreshments, which were provided by the Owl Café, part of the Papworth Trust in support of learning disability.

The age-and-gender matched control group and older control group were principally identified as family members and/or carers of the individuals with DS who were taking part. After initial recruitment through this method, emails were sent to University of Cambridge students and staff to increase participation of particular age-groups. People who were interested contacted the researcher by email to express interest in the study.

2.5.2 Recruitment procedure

Once a participant was identified, contact was made from the researcher to the participant or their family member or carer by phone. The study was briefly explained and if they were interested an information pack was sent to the participant. This included three information sheets so as to appeal to all levels of understanding; a standard version of the information sheet (appendix B), an easier-read version created using Widgit symbols (communication aid for those with intellectual disability (ID), appendix C), and, a picture booklet with fewer words and images of several aspects of the study (appendix D). Participants and their families were given at least one week to read through the materials before a follow up call was made by the researcher to ask if they were interested in proceeding. If the participant was happy to continue the researcher arranged to visit the participant at their home to discuss the study in further detail, and to check that the participant understood the procedure and

could provide informed consent (for the DS consent form see appendix E). For participants who were not able to give their informed consent a consultee process was initiated. In these cases, either a personal consultee, someone who knew the individual with DS as a friend or family member, or a nominated consultee, for example a doctor or keyworker of the individual, was given an information sheet and a declaration form to sign (personal consultee documents, appendices F and G; for nominated consultee documents, appendices H and I).

Control and comparison group participants were approached either in person or via email. If interested in the study they received an information sheet (see appendix J) and were given the opportunity to ask any questions. If they wished to participate in the study they were asked to sign the consent form (see appendix K).

All participants were aware that taking part in the study was voluntary and that they were able to change their minds and leave the study at any time. The home visit to participants with DS was an important part of the study recruitment procedure, allowing the participant to meet the researcher in a comfortable environment and giving them and their carer the chance to ask questions about the study. Participants were recruited nationally and were required to make a single visit to one of the three testing sites for the retinal examination. Home visits for recruitment and cognitive testing are shown in Figure 2.1.



Figure 2.1 Home visit recruitment map. Red pins refer to participant locations and blue pins to the testing sites.

2.5.3 Capacity to consent

Capacity to provide full informed consent was assessed by the researcher. Participants were given as much time as necessary to make a decision about whether to take part and were encouraged to ask questions. Consent procedure was always undertaken in the presence of a parent or carer whom the participant trusted and who could oversee that there was no coercion. Participants were required to read through the information sheet with the researcher and to answer simple questions regarding the nature of the study, i.e. what would be expected of them, what the risks and benefits were and that they understood that they did not have to take part in the study and could change their minds at any time. If the researcher believed that the participant did not have capacity to provide fully informed consent to the study, but that they had expressed a desire to take part, then a consultee process was invoked.

2.5.4 Inclusion and exclusion criteria

Inclusion criteria

- Diagnosis of DS (DS group)
- At least 18 years of age (DS and age-matched control group)
- At least 57 years of age (older comparison group)

Exclusion criteria

- No active psychiatric or neuropsychiatric illness (other than dementia in the DS group)
- No eye surgery within the last three months
- No diagnosis of diabetes mellitus
- No nystagmus or latent nystagmus
- No diagnosis of current or previous retinal disease
- No retinal detachment or vitreoretinal procedure
- No evidence of severe cataracts

Inclusion and exclusion criteria were checked with all participants ahead of scheduling home visits or study visits.

2.5.5 Sample size calculation

The primary aim of this study is to investigate retinal thickness in relation to AD in people with DS. To achieve this, one of the analysis procedures undertaken compared retinal thickness in people with DS to that of age-matched typically developing controls and examined age-related changes in retinal thickness. Previously completed OCT research in people with DS is sparse, the largest study in the literature included 17 children with DS and 18 age-matched typically developing children (O'Brien et al., 2015). This study provided acceptable power of 0.80 and permitted detection of $\geq 10\mu\text{m}$ inter-ocular difference in retinal thickness. In addition to this study, literature in the non-DS AD population was assessed in order to establish the necessary parameters for the current study. Analysis of studies comparing RNFL thickness in patients with AD compared to controls (shown in appendix L) was conducted in order to determine the sample size for this study.

Sample size for a (two-tailed) two-sample t-test comparison was calculated. Given the comparisons identified in the literature we conservatively assume an effect size of $D=1$, along with $\alpha=0.05$ and power=0.9 and calculate a sample size of 23 participants per group (using G*Power version 3.1.9.2, as described in Faul, Erdfelder, Buchner, & Lang, 2009). Given the use of the non-parametric Mann-Whitney U test in Moschos et al., (2012), we inflate this sample size to 24 per group (based on the asymptotic relative efficiency of $3/\pi$ of the Mann-Whitney compared to the t-test, as reported in Lehmann, 1975: $23/(3/\pi)\approx 24$). Finally, we assume a drop-out/loss of information of 20% indicating a need to recruit a minimum of 30 people per group ($30/0.8\approx 38$), giving a total recruitment of 90 people.

In summary, this study aimed to recruit a minimum of 30 participants with DS, aged 18 years and older with no upper age limit, and a minimum of 30 non-DS control participants inclusive of expected dropout rates. As an additional component, 30 typically developing participants older than the DS group were recruited as a comparison group. This older group were combined with the age-matched typically developing controls to create a larger age range for increased understanding of natural ageing changes in typically developing people in certain analyses. The recruitment need was re-assessed as data collection progressed, due to the number of failed scans in the DS group recruitment was increased to account for this in order that the study achieve acceptable power.

2.6 Optical coherence tomography

2.6.1 Equipment

Camera model Spectralis Heidelberg Retinal Angiography + OCT (Spectralis OCT; S3600) was used for all retinal examinations in this study (see Figure 2.2). Heidelberg Eye Explorer version 1.9.10.0, Heidelberg Engineering, Spectralis Viewing Module version 6.3.4.0 (Heyex) software was used to view and process the data. Images were automatically centred, averaged and segmented as part of this software. Segmentation was manually assessed for each participant and corrected where necessary by the researcher using the Heyex viewing module tools under the guidance of Dr Normando. From Heyex, retinal thickness values were extracted manually into statistical programming software IBM SPSS Statistics.

During scanning the automatic retinal tracking (ART) mode was enabled in this camera which ensures that scans are acquired comprehensibly without excessive artefacts from eye

movement. In addition, this camera includes TruTrack eye tracking technology. TruTrack creates a reference image of the eye based on the location of the blood vessels and forces the scan to continue only when the eye was correctly aligned. This greatly reduces artefacts caused by movement in the data, but can increase the length of the scan. Pupil dilation was not required for participants in this study due to the high-resolution capabilities of the camera model used.



Figure 2.2 Heidelberg Spectralis OCT machine used in this study.

2.6.2 Operational procedure

Optical coherence tomography is a medical imaging technique using light to capture micrometre-resolution images from within the biological tissue of the eye. This technology is based on interference between signals from the object under investigation and the reference signal (Podoleanu, 2012). OCT uses low-coherence interferometry to produce a two-dimensional image of optical scattering from the internal tissue (Huang et al., 1991). The scanning resolution of OCT is similar to that of a confocal microscope and is limited only by the coherence length of the light source. OCT resolution is superior to that of both MRI and ultrasound as it depends on light, rather than soundwaves or radio waves, which both have more interference. OCT can maintain a high-depth resolution even when the light opening is small, which is particularly beneficial in the in-vivo measurement of deep tissue.

Light is transmitted to the eye through the use of an interferometer, an investigative tool which merges two or more sources of light to create an interference pattern. The infrared light is transmitted through the pupil; this then penetrates through all the transparent layers of the retina. The light scatters and returns through the pupil, from which the detectors can analyse the interference of light returning from the layers compared to the pattern of light returning from a reference path, such as a mirror (see Figure 2.3). During OCT, the interferometry is able to record the optical path length of the photons received, enabling clear 3D images of samples by rejecting the background signal whilst collecting light received directly from the surfaces of interest.

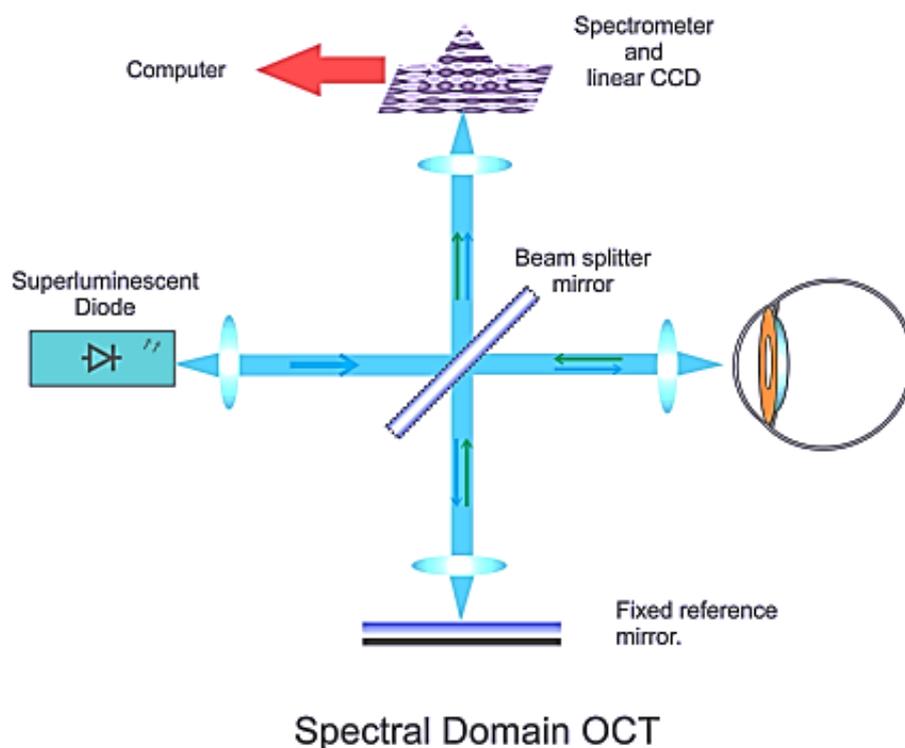


Figure 2.3 Diagram showing the working principle of OCT (image reproduced from Yolcu, Faruk Sahin, & Gundogan, 2013, page 24).

In the medical community OCT is a well-established technique for obtaining images of the anterior segment of the eye and the retina, and is often used for assessing axonal integrity in MS (Dorr et al., 2011), macular degeneration (Keane et al., 2012) and more recently, glaucoma. Advantages of OCT technology include; safety, ease of administration and low cost. OCT has both high depth and transversal resolution, resulting in high speed acquisition

of images. Advances in technology have encouraged its development in a variety of applications, including research, but medical applications remain dominant.

2.6.3 Considerations in the use of optical coherence tomography technology

Several studies have examined the reproducibility and repeatability of OCT technology in reference to the assessment of RNFL measurements in normal healthy participants, as well as in patients with glaucoma, retinal diseases and hypertensive eyes. Overall, a very high intra-class correlation coefficient (ICC) has been identified by these studies, ranging between 0.9 and 0.99 indicating very high reliability between scan measurements (Carpineto et al., 2003; Kim, Yoo, Jeoung, & Park, 2015; Lee, Kim, Jo, & Kim, 2015; Leung et al., 2008; Leung et al., 2009). Studies specifically undertaken to provide repeatability measures of the segmentation process of the retinal layers have also found very positive results. Çetinkaya, Duman, Duman, & Sabaner, (2017) conducted an inter-examiner study where 60 healthy eyes were measured four times by two independent examiners, the repeatability and reproducibility measures for the segmentation of the RNFL and retinal layers were very high (ICC >.96 in all areas) and the study concluded that it was “virtually impossible” to distinguish between measurements taken by different examiners. In individuals with cognitive impairment, Loh et al., (2017) showed a high inter-visit reproducibility in the RNFL and inner retinal layer thicknesses of cognitively stable patients with AD and MCI over the course of one year.

There are many artifacts which can affect the measurements of RNFL thickness, these are critically important to identify and resolve as misinterpretation of the OCT results can lead to misdiagnosis of diseases. There are several factors which can influence the thickness of the retina as reported by both OCT and histology. Individual differences in ocular features and diseases are discussed in this section, followed by a summary of some of the more common features and those which specifically affect the OCT measures.

Individual differences

The size of the optic disc has been found to influence retinal thickness, studies have shown that individuals with larger and more myopic eyes had greater mean RNFL thickness (Bundez et al., 2007; Savini, 2005). Histological studies in monkeys have also shown

increased numbers of axons in those with larger optic discs (Quigley, Coleman, & Dorman-pease, 1990), although this has not been replicated in human samples (Varma, Skaf, & Barron, 1996). Aside from the increased number of axons creating a thicker RNFL, Bunde et al., (2007) also theorised that the increased thickness seen in OCT could be created by the fixed circular RNFL scan which may show an artificially thicker measure if the retinal axons are closer to, or at a different incident plane to, the scanning beam. Macular pigment, which plays a critical role in protecting the macular from harmful blue light, may also artificially increase retinal thickness. Liew et al., (2006) reported that optical density of the macular pigment has a significant positive relationship with the retinal thickness as measured by OCT ($r = .30$). Later studies have supported this, finding that specifically the foveal region of the macular shows increased thickness with high density of macular pigmentation (van ver Veen, Ostendorf, Hendrikse, & Berendschot, 2009). These studies were completed in healthy control participants, in people with DS, three case studies have reported pigmentation diseases in people with DS. In one study a 47 year old male with DS was diagnosed with bilateral congenital hamartomas of the retinal pigment epithelium, a rare anomaly which results in jet black lesions which cover the full retinal thickness of the macular areas (Alexander & Ramirez-Florez, 2008). The second and third case studies were of DS individuals of a younger age, a 12 year old male and a nine year old female (Hayasaka & Hayasaka, 2004; Yamaguchi & Tamai, 1990). Both of these patients had bilateral congenital macular coloboma that included pigment clumps at the macular. Additional presence of other ophthalmic diseases was not reported, and these studies did not report on the patients' retinal thickness. Presence of plaques containing $A\beta$ in the retina is another feature which may increase retinal thickness, and which is particularly relevant to AD studies. Although $A\beta$ plaques have been identified in both mouse and human retinas, there are no studies which have investigated the relationship between burden of retinal amyloid and retinal thickness measures. Studies which have correlated retinal thickness with cerebral $A\beta$ have shown there to be decreased retinal thickness with increase of cerebral $A\beta$ (Golzan et al., 2017). Some of the more commonly occurring artifacts in OCT are recognised below as well as attempts made to resolve these issues.

Age

The age of the patient affects the retinal thickness observed. As previously discussed, there is a significant age-related attrition of the RNFL. Data which does not account for age, or which is subject to errors in the entering of birth dates can implicate the results and lead to erroneous interpretations. In this study age was an important factor and all attempts were made to account for and correctly enter this information.

Signal strength

A reduction of signal strength usually occurs from a media opacity and this can lead to a loss in retinal layer features and artifacts in layer segmentation processing. Most common causes of reduced signal strength include dry eye, corneal opacities, cataracts and vitreal opacities. Studies have shown positive relationships between signal strength and RNFL thickness in healthy patients, amounting to as much as a RNFL thickness decrease of 2 μ m per unit of signal strength decrease (Vizzeri, Bowd, Medeiros, Weinreb, & Zangwill, 2009). Opacities were controlled for in this study and those with severe cataracts were excluded.

Signal quality

The quality indexes of the OCT scans were assessed for inclusion purposes. This scale ranges from 0-40 and the threshold for acceptability is >15 (Huang et al., 2012). In this study the signal strength of all retained scans was deemed to be acceptable. Those with a quality index <15 were excluded from the study and data was not entered into any analyses.

Improper alignment

Incorrect alignment of the OCT images can lead to segmentation errors and artificial thickening or thinning of the retinal layers. This is majorly a problem during repeat scans and can introduce variability if the alignment is not precise. The SD-OCT TruTrack technology has worked to vastly reduce this issue. Alignment errors can also occur during the automatic segmentation procedure however, therefore it is necessary to check and manually re-centre the scans if necessary. De-centring of the image can cause truncation, which reduces the thickness reported by the OCT. This problem is emphasised in patients

with disc edema, tilted optic nerves or significant cupping as the RNFL is difficult to capture on a single B scan due to varying heights, however it is still a relevant factor for all patient and healthy groups (Chen & Kardon, 2016).

Segmentation errors

Heyex viewing module software provides automatic segmentation of the retinal layers and automatically centralises the scan to either the optic nerve head (ONH) or the fovea, depending on the particular scan. Segmentation errors can be influenced by poor signal strength and various ocular diseases that were controlled for in this study (Ho et al., 2009). Prior to analysis all scan segmentations were checked and manually adjusted where necessary after consultation with the lead ophthalmologist.

Axial length

Ambiguous findings are sometimes presented during OCT scans due to the shape of an individual's eyes. Shorter eyes typically present with thicker RNFL values due to camera magnification, whilst longer eyes can cause artificially thinner RNFL measures (Kaliner, Cohen, Miron, Kogan, & Blumenthal, 2007).

Saccades

Excessive eye movement or blinking during the scan can cause incorrect alignment errors leading to erroneous measures which can be misinterpreted as progressive thinning. The OCT camera model used in this study has a built-in eye tracking function, TruTrack, which greatly compensates for eye movement by relying on blood vessel registration or iris tracking. The use of the eye tracker significantly improves the reproducibility of RNFL measurements (Langenegger, Funk, & Töteberg-Harms, 2011).

Heyex normative database

The Heyex viewing module software includes a normative database that is age-matched to the individual undergoing examination. The normative database consists of 284 healthy individuals with an age range of 18-84 years (mean= 46.5 years). Ethnicities include; 43%

Caucasian, 24% Asian, 18% African American, 12% Hispanic, 1% Indian, and 6% mixed ethnicity. The raw data for the normative group is not commercially available; therefore it was necessary to have an additional age-matched control group for making group comparisons between people with and without DS.

The colour grading system used in the Heyex viewing module is representative of the normative database. Retinal thicknesses which fall within the green area are within the 5th – 95th percentile for age, within the yellow region are the 1st -5th percentiles and the red area are those below the 1st percentile (see Figure 2.4).

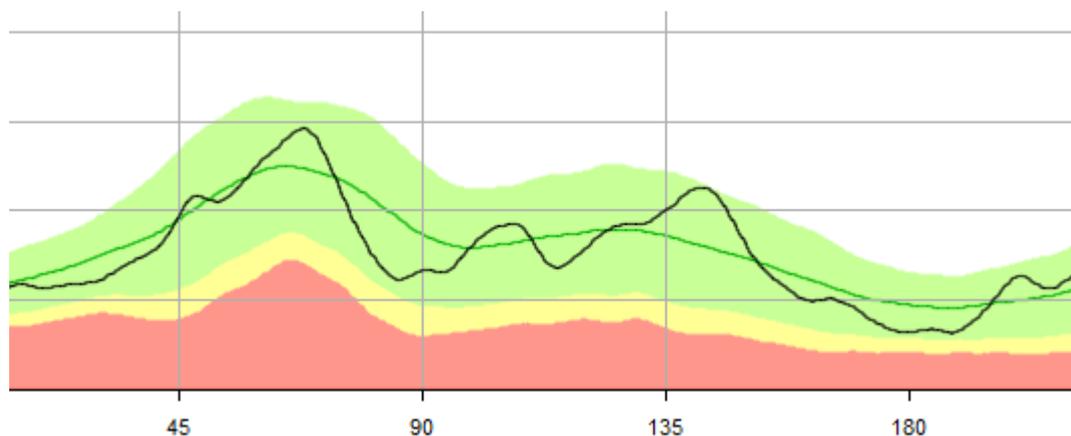


Figure 2.4 Example of the normative database line (shown in green) against the individual participant data (black).

2.7 Retinal scans

Differences in retinal thicknesses between people with DS, age-matched controls and an older comparison group were explored in this study. Three areas of the retina were evaluated, the RNFL, macular, and the retinal layers.

2.7.1 Retinal scanning procedure

All retinal examinations for all participants were completed using the Spectralis OCT and were conducted by Dr Eduardo Normando (University College London and Imperial College Healthcare NHS trust). Informed consent was gained before scanning commenced and the procedure was fully explained to the participant. Retinal scans were conducted at one of three locations;

- Douglas House, Department of Psychiatry, Cambridge University
- ICORG Unit, Western Eye Hospital, London
- Down's Syndrome Association Headquarters, Langdon Down Centre, Middlesex

The OCT scanner is set up on an adjustable table. After the participant is seated the OCT table is moved towards them until they can comfortably reach the chin rest. The chin rest is aligned dependent on eye level and the forehead rest adjusted to suit the participant. The camera is manually positioned in front of the selected eye and the participant is asked to look inside the camera lens at a blue light, if they are unable to see the light, an external fixation point (an adjustable arm with a light) was used to attract the participants gaze. Once the participant had fixated on the light they were asked to keep looking straight ahead and to try not to move their eyes. Participants were reminded that they could blink, but to look straight ahead at the light afterwards, and to ignore any red lights they may see within the camera.

Two scans were completed for each eye, four scans in total. If necessary, scans were repeated an additional one time each if the signal quality was low, highest quality scans were retained. Scans were not completed sequentially across participants. Breaks were given after each individual scan and additional breaks during the scan if necessary. All scans were completed in one visit, if a participant was unable to successfully complete a scan during the testing visit, that scan was considered lost. Scan procedure lasted approximately 10-20 minutes for the DS participants. Duration of the scans were dependent on the amount of head movement, distractions, the number of breaks the participant needed to take and their individual ability to keep their gaze fixated. Average scan time for the control group was less than five minutes. After the scans were finished the participant was thanked and allowed to leave, there were no after effects of the scan procedure.

2.7.2 Scan sequences

Retinal areas of interest were derived from literature reviews of studies in AD and of the few studies examining retinal thickness in children with DS. Two scan types were invoked in this study, peripapillary retina (referred to as the RNFL scan) and retina (referred to as the posterior pole and macular scans). Thicknesses of these three areas were evaluated. Figure 2.5 shows a digital retinal photograph of the eye and how these three areas relate

within the eye. Macular and posterior pole scans are acquired at the same time using a box scan, from this, data is automatically generated for both regions of the retina.

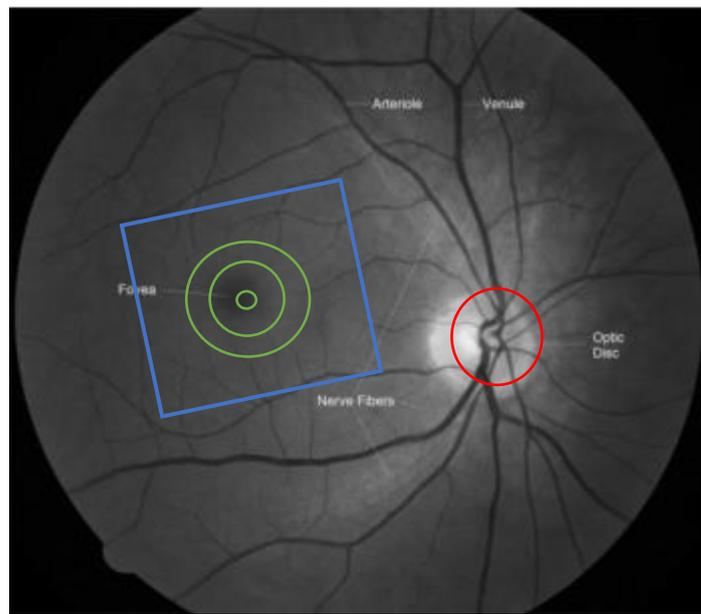


Figure 2.5 Digital photograph of the retina. The RNFL (red circle), inner and outer macular (green circles) and posterior pole area (blue box) assessed in this study have been highlighted (not to scale). Original figure reproduced from Frost, Martins, & Kanagasingam, (2010), page 7.

2.7.2.1 *Retinal nerve fibre layer scan*

The RNFL scan derived the thickness of the RNFL retinal layer in a 12 degree (3.2mm) circumference surrounding the ONH (see Figure 2.6(a)). This scan was centred on the ONH with 30 degree angle. Glaucoma application and high speed resolution and automatic tracking mode (ART) were enabled. ART enables multiple frame scanning of the same location, which are averaged for enhanced noise reduction. Additional data gathered from this scan included scan quality and eye length. OCT provides detailed topography of the retinal layer identified during the scan. Manual adjustments to the line can be made in case of automation malfunctions.

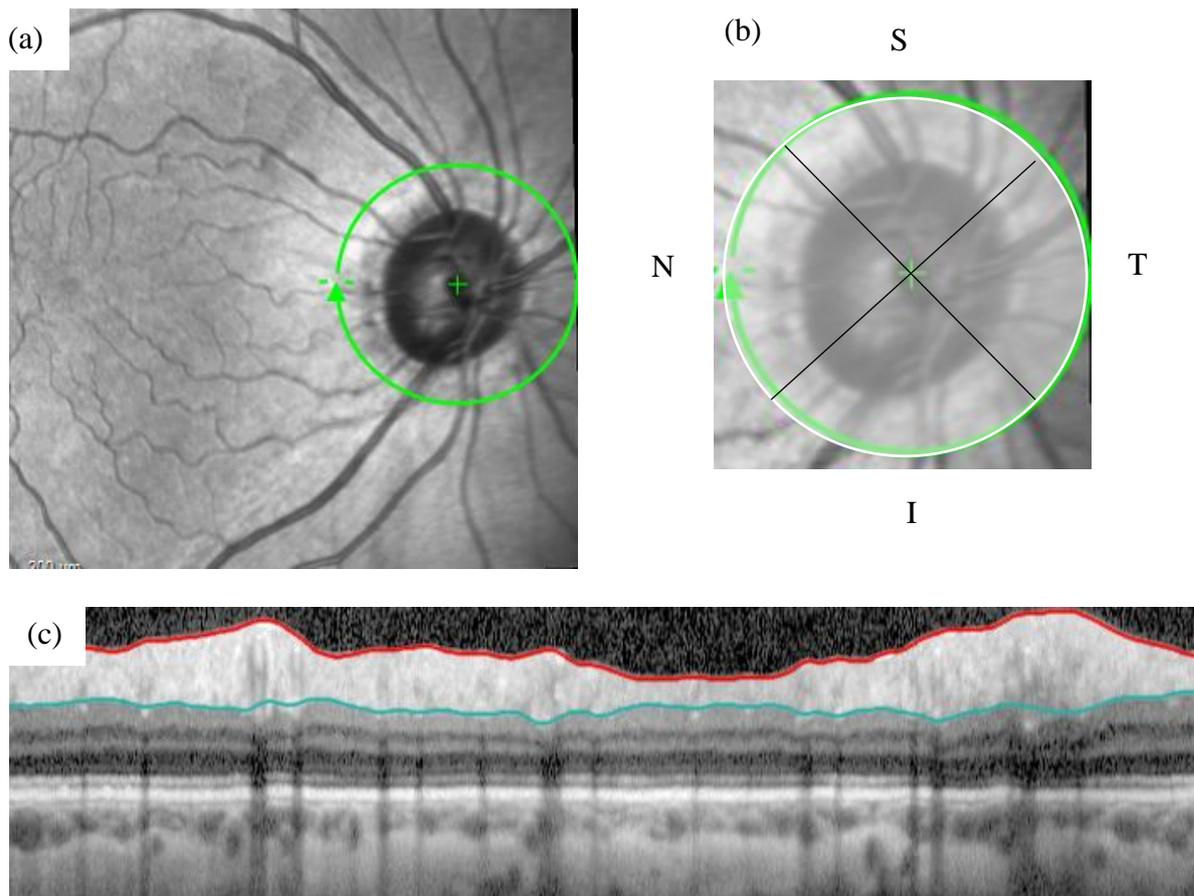


Figure 2.6 Images showing the RNFL images acquired. Figure (a) shows the ONH with the RNFL image line surrounding it. Figure (b) demonstrates the sectioning of the RNFL line with corresponding quadrants; superior (S), temporal (T), inferior (I) and nasal (N). Figure (c) shows the circle stretched into a line and the thickness of the RNFL represented between the red and blue lines.

Data for the RNFL scan is produced and automated averages of each section are given (see Figure 2.7). These measures are compared to the normative database (section 2.6.3) and stratified dependent on thickness classification in comparison to others of their age. Individual values are given in black and the expected value based on the normative database in green. Areas are shown as green (inside normal limits), yellow (borderline), or red (outside of normal limits). It is important to note that “borderline” and “outside of normal limits” refers to thinner values than expected, rather than thicker values.

Heyex automates the average thickness for the global (360°) RNFL and for the six areas within. Of these six areas, the temporal superior and nasal superior values were averaged to

create a single thickness measure for the “superior quadrant” and the temporal inferior and nasal inferior values averaged to create the “inferior quadrant”, as is typical in previous literature. The averages of these quadrants were manually computed. All output values are measured in micrometres (µm).

In addition to the classification chart, a RNFL thickness profile graph (Figure 2.8) is automatically computed. This graph shows the topography of the entire RNFL, rather than the averages of each sector. From this graph, mapping of blood vessels and other retinal features can be accomplished as the area shown in Figure 2.6(b) is the retinal photograph of the same area and is positioned directly above the profile graph on the viewing screen with a connecting reference line.

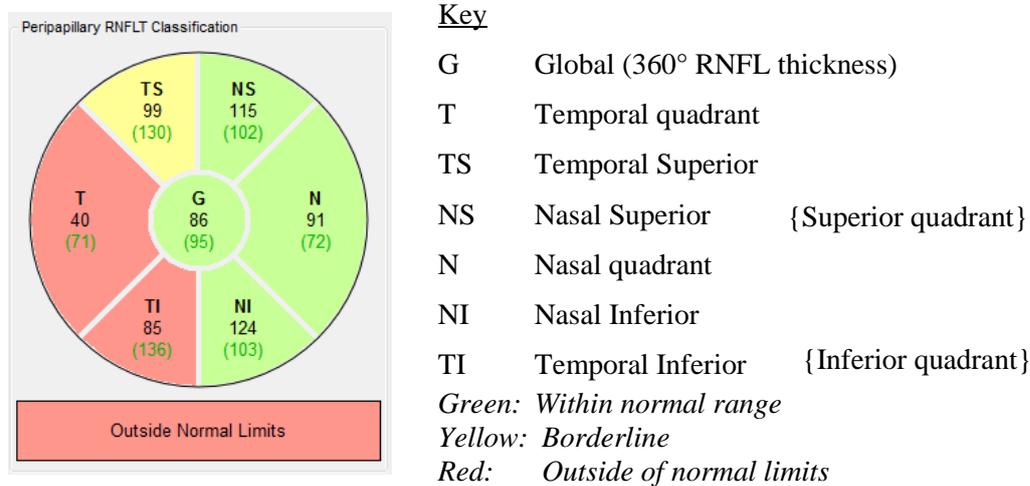


Figure 2.7 Peripapillary RNFL thickness classification chart

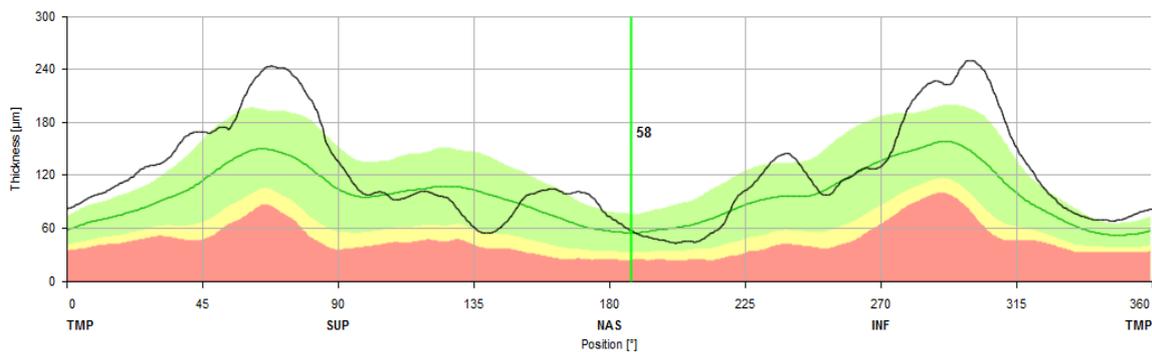


Figure 2.8 RNFL thickness profile graph. Individual participants’ RNFL measure is shown on the black line and compared to the averaged normative data of a typically developing cohort of the same age (green line).

2.7.2.2 Macular scan

The area covered in the retinal box scan comprises the macular and the posterior pole, this scan is centred on the fovea. Throughout this thesis the macular and posterior pole data will be referred to separately. The retina scan uses high speed resolution with retina application and a scan angle of 30 degrees. ART mode was enabled and eye length was recorded. Scan quality is not automatically generated, however each of the 61 scan lines that make up the box scan have an individual quality index number.

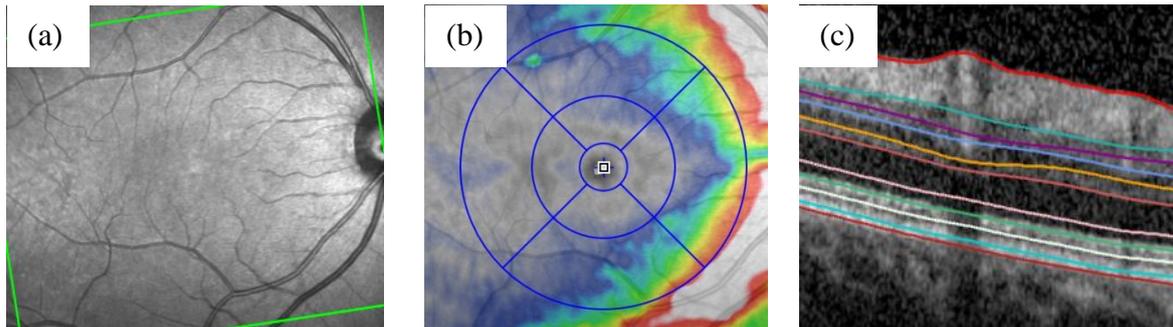


Figure 2.9 Examples of images produced for macular and retinal layer data. (a) box scan area centred on the fovea, (b) macular region within the box scan and, (c) segmentation of retinal layers.

The macular region is categorised into three areas; the fovea (1mm ring), the inner macular (3mm ring) and the outer macular (6mm ring; see Figure 2.10). Each of the inner and outer macular rings are subdivided into quadrants (superior, inferior, nasal and temporal; see Figure 2.11). In this study the fovea, inner and outer macular averages are evaluated first; additional analyses will consider the quadrants of the inner and outer rings if appropriate. As with the RNFL scan, macular data is averaged between the two eyes to provide one value composite for each region for each participant.

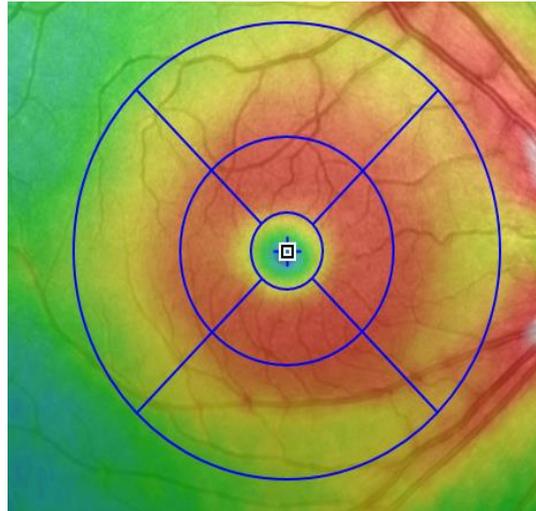


Figure 2.10 Automated macular thickness colour map. Warmer colours represent thicker areas and cooler colours reflect thinner areas. The three circles represent macular areas; smallest (1mm) ring is the fovea area thickness, inner macular area is within the second ring (3mm) and the largest ring is the outer boundary of the outer macular area (6mm).

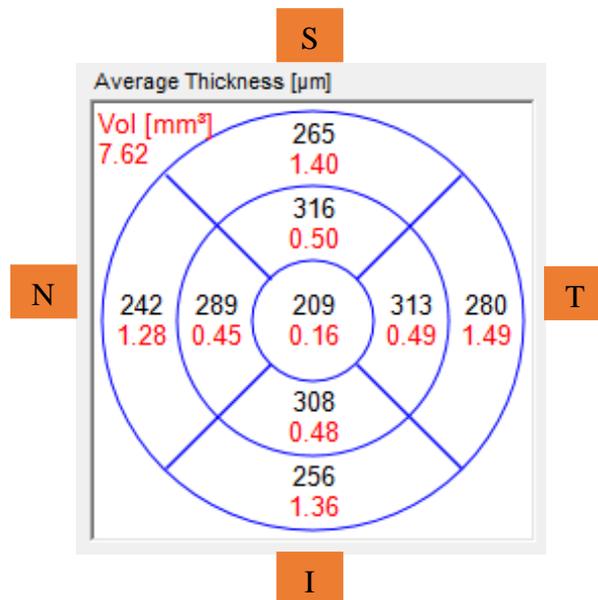


Figure 2.11 Example of macular scan output and quadrant division. Thickness measures for each region are shown in black and volume in red. Quadrants include the superior (S), temporal (T), inferior (I) and nasal (N).

2.7.2.3 Posterior pole scan

The posterior pole scan comprises the area surrounding the macular up to the optic disc. This scan is also centred on the fovea but encompasses a larger area than the macular ($30^\circ \times 25^\circ$), as shown in Figure 2.12. Each retinal layer has a colour grading system detailing thinner and thicker retina. As with the macular, warmer colours represent thicker retina and thinner retinal measures are in cooler colours. Each box within the 8x8 grid shows the mean thickness value for that area.

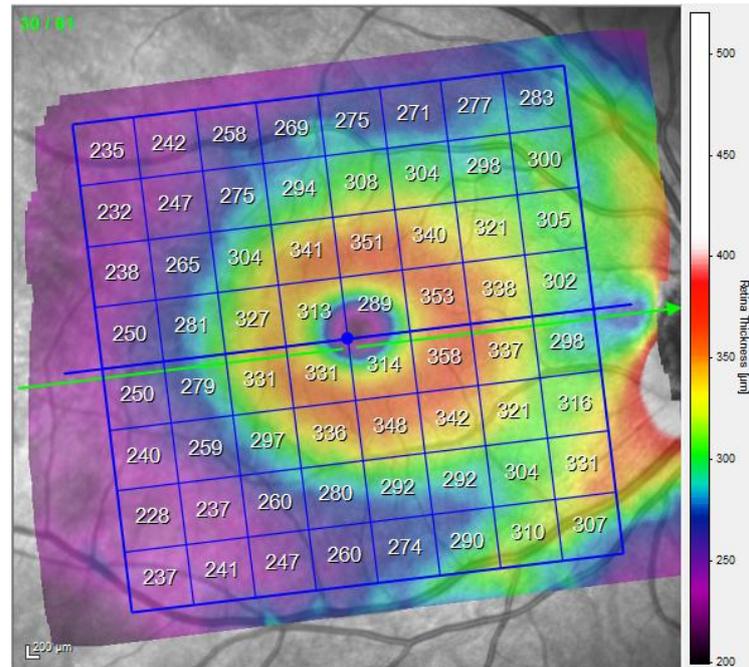


Figure 2.12 Posterior pole retinal scan. This images shows the average thickness of all retinal layers. The colour scale shows thicker retina in warmer colours and thinner areas in cooler colours.

After acquisition, all data was quality screened and pre-processed, including manually centred and adjusted where necessary. Segmentation processing was performed on all scans to delineate the retinal layers (see Figure 2.13). Further exploration of the images was completed to confirm correct segmentation and adjusted if necessary. The layers of the retina are divided into those which make up the inner layers and those which make up the outer layers. Inner layers consist of the nerve fibre layer (NFL) to the INL and outer layers consist of the outer plexiform layer (OPL) to the RPE.

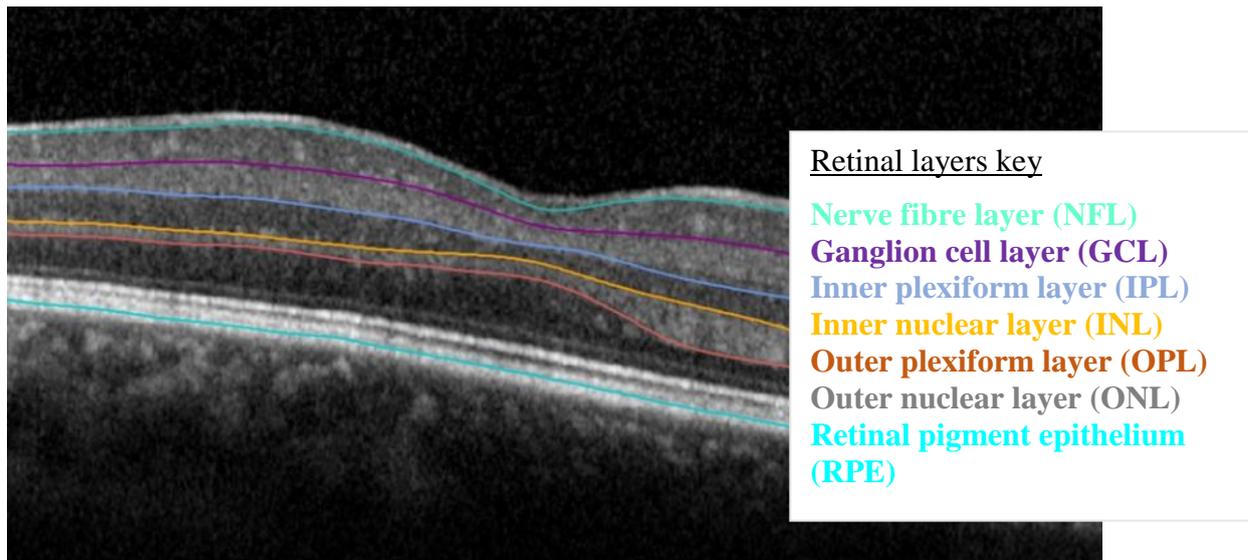


Figure 2.13 Segmentation of the retinal layers within the posterior pole scan.

Presentation of retinal data varies significantly within the published literature. Some studies have chosen to present the data from eye scans independently, reporting both a left and right eye, randomly chosen either the left or the right eye, pre-selected either the left or right eye only, averaged data from both eyes and presented one set of values. This last methodology has been chosen for this study when presenting the data of the RNFL and macular regions. Before deciding on this method paired comparisons were conducted for all retinal regions which showed that overall there were no significant differences between the measures of the left and right eyes. Data for these comparison tests can be seen in appendix M.

For the posterior pole data, each of the seven retinal layers, plus the average of the inner layers, outer layers and total layers for both eyes, amounts to a total of 1280 data points per participant that would need to be manually extracted and averaged. Based on statistical and ophthalmological expert guidance it was decided to present the data of the eye with the highest scan quality image. Quality index in this scan is reported for each scan line, a total of 61 for one eye. An average of these 61 quality indexes for each eye was calculated to find the overall quality index and compared to the overall quality calculation for the other eye. The eye with the best overall scan quality was retained and entered into analyses. Full details of this procedure can be seen in appendix N.

2.8 Neuropsychological evaluation

Associations between IQ, cognitive performance and dementia status were assessed in this study. A neuropsychological evaluation for the participants with DS was undertaken, comprising an IQ test (KBIT-2; Kaufman Brief Intelligence Test, Kaufman & Kaufman, 2004), and two cognitive function batteries; the Cambridge Cognition Examination (CAMCOG) and an executive function battery. In addition, a dementia screening interview, the Cambridge Examination for Mental Disorders of Older People with Down's Syndrome and Others with Intellectual Disabilities (CAMDEX; Roth et al., 2014), was conducted with the DS participants' main carer.

For the control and comparison group participants a dementia screening test, Telephone Interview for Cognitive Status (TICS; Brandt, Spencer, & Folstein, 1988) was administered, to rule out any possibility of dementia. Neuropsychological measures and the informant dementia interview can be found in appendices P-R.

2.8.1 Procedure

Neuropsychological tests were conducted to provide a comprehensive cognitive assessment for the DS participants. These tests were conducted either at the testing site or the participants' homes. Testing procedure ranged between 1-2 hours and was completed over one or two visits depending on the participant's preference. Assessments completed in this study are shown in Table 2.1.

Assessments completed	Estimated time (mins)
Participants with DS	
CAMCOG (Ball et al., 2004; Hon, Huppert, Holland, & Watson, 1999)	30
Selected tests from the Cambridge Executive Functioning Assessment (Ball et al., 2008)	30
KBIT-2 (Kaufman & Kaufman, 2004)	30
<i>Assessments completed by parent/carer</i>	
CAMDEX informant interview	45
Control and comparison group participants	
Telephone Interview for Cognitive Status (Brandt et al., 1988)	5

Table 2.1 Neuropsychological assessments completed.

2.8.2 Neuropsychological tests

2.8.2.1 CAMCOG

The CAMCOG (Ball et al., 2004) was designed to assess cognitive impairment characteristics of the general elderly population with reference to dementia. Minor modifications have been made to the CAMCOG in order to increase its suitability for people with DS. CAMCOG for use in people with DS incorporates the MMSE and can be used to determine a global estimate of ability, Spearman's rank correlation between test scores of both measures showed a correlation of 0.96 after exclusion of identical items (Hon et al., 1999).

The CAMCOG is divided into the following areas of cognitive function: orientation, language, memory, attention and calculation, praxis, abstract thinking and perception. Some of these categories are then further divided, i.e.; language is split into comprehension (motor response, verbal response and reading) and expression (naming, fluency, definitions, repetition and writing) and memory is divided into remote memory, recent memory, incidental learning and intentional learning. The subscale scores for the seven categories are summed for an overall CAMCOG score with a maximum of 109 points.

Examples of questions from the CAMCOG include; (1) "What is the name of the princess who died in a car crash in Paris?" if no response is given the examiner may follow with a

prompt, i.e. “She was married to Prince Charles”, (2) “Please nod your head”, (3) “Copy this shape in the space below”. For the full CAMCOG questionnaire and score sheet see appendix A.

2.8.2.2 *Kaufman Brief Intelligence Test 2*

The Kaufman Brief Intelligence Test, second edition (KBIT-2; Kaufman & Kaufman, 2004) is a test for approximate IQ consisting of a verbal and a non-verbal component. This test is copyright protected and therefore a full version cannot be made available in the appendices. For verbal knowledge, a single word is delivered by the examiner to the participant, with a choice of six images from which the participant selects the image which best represents the word given. In each case there is only one correct answer and no further explanation or assistance can be given by the examiner. The second part of the verbal knowledge score is a “riddles” test. Here the examiner describes an object or concept and the participant has to give the name of what is being described. For example, the examiner would say “What hops, eats carrots and has long ears?” and the participant could answer with one of the correct answers “rabbit” or “bunny” or an incorrect answer. Examples of correct and incorrect answers are given for each riddle; in this case an example incorrect answer would be “kangaroo”. Each section of the test has a floor and ceiling score and the test is stopped when four consecutive incorrect answers are given. In between the two verbal components, the non-verbal component is assessed using matrices, such as choosing which pictures go together, which picture is the odd one out, and which picture fits. There are training items during this test to prepare participants for the next set of questions. For example, an incorrect response in Figure 2.14 would result in the examiner saying, “The rabbit’s ears look like this (point to rabbit’s ears) and the elephant’s ears look like this (point to elephant’s ears). So these ears go with the elephant. Let’s do it again”.

Non-standardised scores from the KBIT-2 were analysed in this study. Standardised scores are calculated using the individuals age, in people with DS this is not appropriate as mental age in DS is often considerably lower than actual age and would dramatically increase the floor effect of the test.

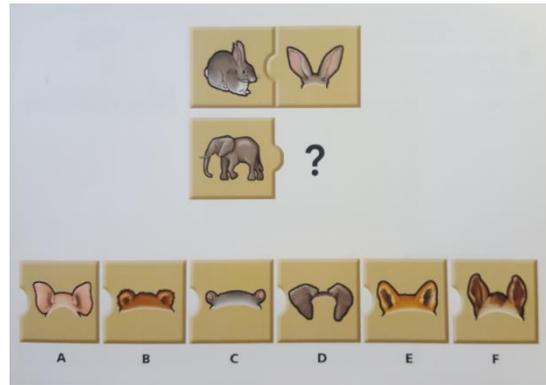


Figure 2.14 Example of non-verbal matrices question in KBIT-2

2.8.2.3 *Executive function battery*

The executive function battery for people with ID (Ball, Holland, Treppner, Watson, & Huppert, 2008) uses a variety of short memory tests including the following tasks as detailed below. A maximum score of 56 is achievable on the executive function battery, for the full scoring sheet and procedures see appendix Q.

Cats and dogs task

The cats and dogs task (Willner, Bailey, Parry, & Dymond, 2010) measures response inhibition and working memory. Initially participants are timed on naming cats and dogs correctly, then training is given to name dogs as cats and cats as dogs. Responses are again timed and the difference between first and second times recorded.

Spatial reversal task

This task measures spatial memory and response inhibition. At the start of the task two coins are placed under two boxes, and the participant is asked to guess where the coin is. At this stage the participant is always correct and whichever box the participant chooses the other coin is removed. The examiner then places a screen in front of the box and pretends to, but does not, move the coin. The participant needs to correctly find the coin, in the same place, four times before the coin is moved to the other box. At which point, they are scored on consecutively choosing the correct (same) box four times.

Tower of London Task

The Tower of London task (Figure 2.15; Krikorian, Bartok, & Gay, 1994) measures planning ability and working memory using a peg board and three coloured beads. The aim is to move the beads to the correct position in the fewest number of moves, whilst remembering to only move one bead at a time. Participants are given three attempts to complete the task and points awarded are reduced at each try.

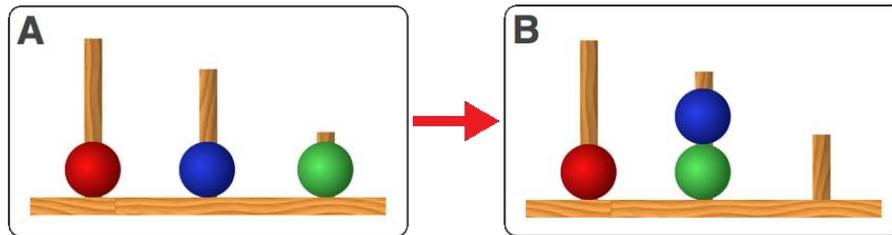


Figure 2.15 Example of the Tower of London task

Weigl sorting task

This task measures conceptual learning. The participant is presented with nine shapes, three circles, three squares and three triangles. One of each shape is coloured red, blue and yellow. Participants are asked to sort the items; correct sorts are by colour or shape. The items are then mixed up and the participant is asked to sort them a different way, to score maximum points the participant must sort the shapes the other way first time.

Scrambled boxes task

This task measures working memory and response inhibition. To complete this task the participant must correctly find three coins within three, and then six, boxes. Each box has a different shape on the top and the participant must attempt not to open boxes they have already checked and attempt to remember which boxes the coins have been placed in. On the first trial boxes stay in the same position, on the second trial they are moved/scrambled by the examiner.

2.8.3 Diagnosis of dementia

2.8.3.1 CAMDEX

Dementia status was assessed in people with DS using the Cambridge Examination for Mental Disorder of Older People with Down's Syndrome informant interview (CAMDEX; Ball et al., 2004; Holland et al., 1998; Roth et al., 2014; see appendix R). Questions are designed to give a detailed overview of a person's memory, general mental and intellectual functioning, general performance, judgement, specific higher cortical functions, personality, presence of specific symptoms and relevant previous medical history. The CAMDEX has been slightly modified to better address the concerns of individuals with an ID, taking into account the pre-existing impairment of cognitive and functional abilities. Most questions have a follow up query of "Is this a change?" or "Is this a deterioration?" and then "Slight or great deterioration?" to address this. The CAMDEX informant interview assesses a wide range of functions including; education, employment, basic skills (speech, understanding, reading, writing and basic arithmetic), independent living skills and any deterioration seen in any of these categories.

2.8.3.2 Telephone Interview for Cognitive Status

For control participants dementia status was assessed using the TICS (Brandt, Spencer, & Folstein, 1988). This test is copyright protected and therefore a full version cannot be made available in the appendices. The TICS was developed as a telephone alternative to the MMSE (Folstein et al., 1975), which is one of the most widely used screening instruments for the detection of cognitive impairment (Nelson, Fogel, & Faust, 1986). The TICS aimed to solve the problem of participant dropout rate due to inability or reluctance to return for in-person assessment. This would also increase cost-effectiveness for large-scale studies. TICS correlates highly with the MMSE $r = 0.86$ ($p < .001$) and has high test-retest reliability, and high sensitivity and specificity for cognitive impairment in a clinic sample of AD patients (Järvenpää, Rinne, & Rähä, 2002).

2.9 Brain imaging

In the final results chapter (chapter five) exploratory analyses are conducted to examine the relationship between retinal and cortical thickness measures, and retinal-cortical thickness relationship differences based on the presence of fibrillar A β binding in the brain. These analyses were undertaken on a reduced sample of the participants with DS included in the retinal imaging study (n=18). Cortical thickness was assessed using MRI and brain A β binding was assessed using Pittsburgh compound [¹¹C] (PIB) and PET imaging approximately two years prior to the retinal imaging study. Cortical thicknesses and binding data were collected and analysed by collaborators with the project. All retinal thickness data comparisons with imaging data were completed as part of this PhD.

2.9.1 Procedure

2.9.1.1 MRI neuroimaging

Adults with DS underwent a 45 minute structural MRI scan on a 3-Tesla Siemens MAGNETOM Verio scanner (Siemens AG, Germany). Acquired MRI data were used for co-registration to the PET data and to detect regions of interest. The imaging protocol included whole-brain, T2-weighted, half-fourier acquisition, single-shot turbo spin echo sequence to assess for vascular pathology and incidental lesions. Full methodology of the MRI imaging acquisition can be seen in Annus et al., (2017).

Average measures from the cortical regions of interest were gathered from the MRI imaging data. Cortical surface analysis was performed in FreeSurfer (version 5.3; available from <http://surfer.nmr.mgh.harvard.edu/>). FreeSurfer calculates cortical thickness using an estimate of the width of the cortical grey matter (the procedure used for cortical thickness analysis is described in; Dale, Fischl, & Sereno, 1999 and Fischl & Dale, 2000). FreeSurfer automates hemisphere-specific data of the cortical surface together with individual participant atlases to enable anatomical localisation. Cortical thickness represents the closest distance between white matter surface (grey and white matter boundary) and pial surface (grey matter and cerebrospinal fluid boundary) at each vertex within the surface model (see Figure 2.16). Cortical maps are not restricted to the volumetric MRI voxel resolution and can be modified to create an individual, but comparable, surface coordinate system.

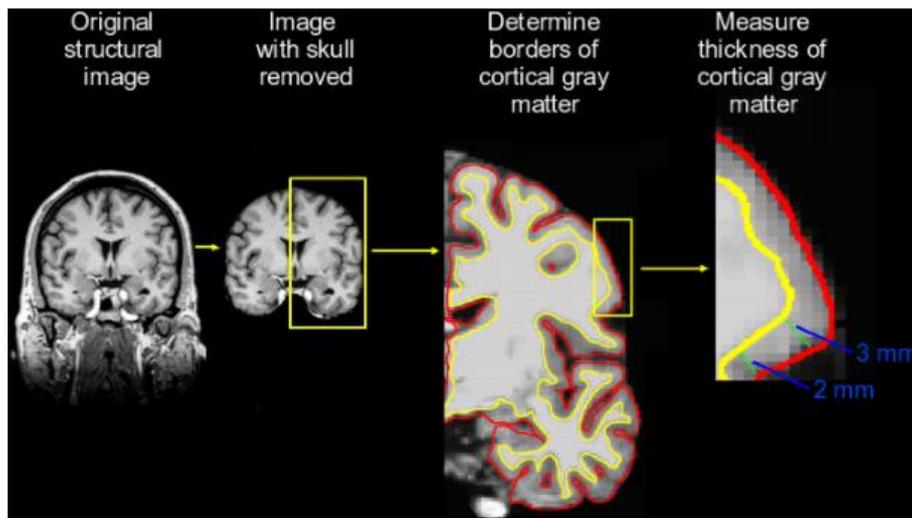


Figure 2.16 Schematic showing cortical thickness calculations. Measurements are taken from between the pial surface (red line) and the white matter surface (yellow line). This figure has been reconstructed from FreeSurfer tutorial materials (available at <https://surfer.nmr.mgh.harvard.edu/fswiki/Tutorials>).

2.9.1.2 PET imaging

Participants with DS underwent a 90 minute dynamic PET scan with [^{11}C]-PIB (produced at the WBIC radiochemistry laboratory with high radiochemical purity (>95%) and specific activity (>150 GBq/ μmol) under Good Manufacturing Practices conditions). Full details on data acquisition have been published previously (Annus et al., 2016). Scan data was acquired in 3D mode on a General Electrical Medical Systems advanced PET scanner. Binding potential maps were created using the simplified reference tissue model (Gunn, Lammertsma, Hume, & Cunningham, 1997). Grey matter tissue from the cerebellum was used as reference tissue. The cerebellum was chosen as the reference tissue as per the pilot study (Landt et al., 2011), and as the cerebellum is relatively free of fibrillar A β (Lalowski et al., 1996; Mann, Jones, Prinja, & Purkiss, 1990). Statistical parametric mapping, version 8 (www.fil.ion.ucl.ac.uk/spm/software/) was used to co-register the mean realigned image to the MRI and manually draw regions of interest. [^{11}C]-PIB PET was used to investigate the load and distribution of fibrillar A β in people with DS and to assign adults with DS to A β positive and negative groups. All brain imaging data was collected by radiographers at the WBIC. Scans were examined by a neurologist at the WBIC and analysed by WBIC physicists together with research assistants from the CIDDRG. No pathology outside of AD-related atrophy was reported.

Mean cortical A β load was calculated for each participant by taking a mathematical average of the non-displaceable binding potential (BP_{ND}) data across all cortical regions of interest. Reconstruction and the kinetic modelling of the raw PIB data were conducted by Dr Hong and Dr Fryer at the WBIC, University of Cambridge. A positive region was defined as having binding potential two standard deviations above that of the PIB negative group. This study also included 10 age-matched control participants to ensure that measures deemed PIB negative in the DS population would also be considered negative in the general population.

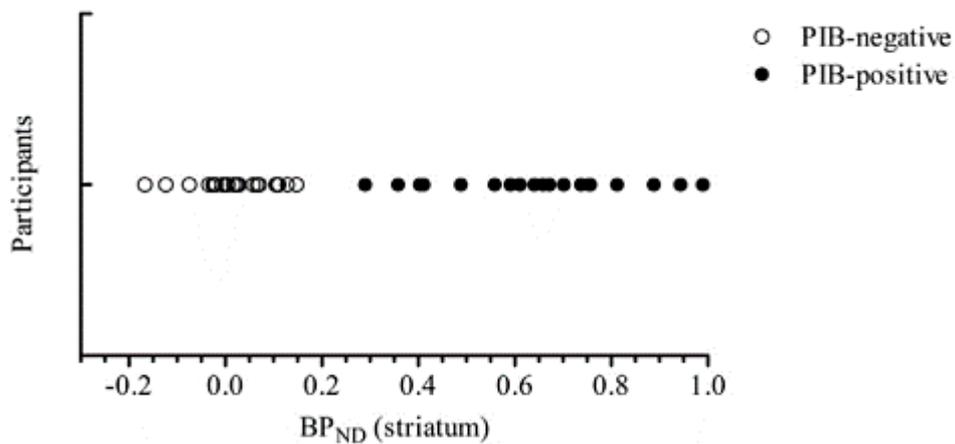


Figure 2.17 Scatterplot representation of the striatal binding potential for all participants included in the DiDS neuroimaging study. Classification of the groups were as either positive or negative. Figure reproduced from Annus et al., (2016), page 541.

2.10 Statistical analyses

Statistical analysis was performed using IBM SPSS statistics 22. Analysis was completed in a top-down method first examining the global value of each measure (global RNFL, fovea, inner and outer macular and inner and outer retinal layers) and then by investigating individual layers and specific quadrants of those areas with significant differences or of particular interest.

All data was tested for normal distribution and outliers using Shapiro-Wilk tests and visualisation of the data, significant values ($p < .05$) were deemed not normally distributed. Outlier sensitivity analyses (repetition of tests with and without outliers) were conducted

where necessary. Independent and paired student t-tests, and ANOVAs were used to compare data and Pearson’s bivariate correlations were used to explore associations between data. Non-parametric tests (i.e. Mann Whitney U tests and Spearman’s correlations) were used when necessary and when tests of normality were not met. Further analyses to compare correlations between groups were conducted using Fisher’s exact correlation tests. Effect sizes were calculated using the below equation as reported in (Rosnow & Rosenthal, 2005).

$$r = \sqrt{\frac{t^2}{t^2 + df}}$$

There were large multiple comparison effects in this study. These were controlled for by applying the Bonferroni correction to the p-values as indicated in previous published literature. Significant p-values to the level of the Bonferroni correction are indicated throughout this chapter with an asterisk. Each of the three retinal regions had a different number of related retinal areas, therefore three different significance levels were used in this study (see Table 2.2). The Bonferroni correction was calculated by dividing the uncorrected p-value (0.05) by the number of related regions within each retinal region. Adjusted p-values were applied throughout all data analyses of all results chapters.

Region	Areas within each region	Total	Uncorrected p-value	Adjusted p-value
RNFL	Global, temporal, inferior, nasal and superior	5	0.05	0.010
Macular	Fovea, quadrants of the inner ring and quadrants of the outer ring	8	0.05	0.005
Posterior pole	NFL, GCL, IPL, INL, OPL, ONL, RPE	7	0.05	0.007

Table 2.2 Table showing the adjusted p-values for different retinal areas based on the Bonferroni multiple comparisons correction.

Chapter Three:

Retinal thickness in adults with Down's syndrome in comparison to typically developing adults

3.1 Chapter introduction

In this results chapter, retinal thickness in adults with Down's syndrome (DS) is considered in comparison to retinal thickness of age-matched controls and older controls, as well as in relation to advancing age. Retinal thickness was measured using optical coherence tomography (OCT) scanning, regions of interest included the peripapillary retinal nerve fibre layer (RNFL), the macular, and the retinal layers of the posterior pole. Previous research in typically developing adults has shown evidence of age-related degeneration in the retina, specifically resulting in thinner RNFL, and is considered part of normal ageing. In patients with AD and MCI the retina shows further thinning compared to age-matched controls.

3.2 Background

The retina is an extension of the central nervous system (CNS) and shares its embryological origin with the brain, as well as many of its anatomical and physiological features (Ikram et al., 2012). As the brain is structurally different in people with DS compared to typically developing people, for example smaller overall brain volume and larger subcortical grey matter volume (Pinter et al., 2001), it is possible that there may also be structural differences within the retina. Previous research has recognised the potential of the retina as a valuable site for visualising changes related to the onset of AD. Despite this, very few research studies have considered retinal structure or thickness in people with DS, and none as part of investigations into dementia.

Retinal thinning is considered to be part of normal ageing (Bundez et al., 2007; Poinosawmy, Fontana, Wu, Fitzke, & Hitchings, 1997). Thinning rates of $-0.16\mu\text{m}$ and $-.38\mu\text{m}$ have been reported in the typically developing population between the ages of five and 90 years, with higher rates of decline seen after the age of 50 years (Parikh et al., 2007; Poinosawmy et al., 1997). Within the RNFL, the superior and temporal quadrants

show the most significant changes (Poinoosawmy et al., 1997; Varma, Skaf, & Barron, 1996), and the inferior quadrant is the most resistant to thinning (Parikh et al., 2007). Normal age-related thinning of the macular has also been reported, predominantly findings have shown the inner macular to be more affected, with an average decrease of $-0.18\mu\text{m}$ per year, in comparison to the outer macular, with an average decline of $-0.03\mu\text{m}$ per year (Garcia-Martin et al., 2016; Iseri et al., 2006). However some studies have found no significant age-related thinning of the macular (Guedes et al., 2003).

Retinal thinning is also established in people with MCI and AD (Garcia-Martin et al., 2014; Kesler et al., 2011; Parisi et al., 2001; Polo et al., 2017), a literature review of this research is presented in section 1.5. The RNFL is the most commonly reported retinal area with significantly thinner values seen in the global RNFL and in the quadrants (Berisha et al., 2007; Gunes et al., 2015). The superior quadrant is the most susceptible to thinning in MCI and AD patients (Eraslan et al., 2015), and unlike in normal ageing, the inferior quadrant is more affected in AD (Cunha et al., 2016) and the temporal quadrant shows the least amount of change (Iseri et al., 2006). Although assessed in fewer studies, the macular also shows significant thinning in AD and MCI patient groups (Cunha et al., 2016; Moschos et al., 2012). Controversially, one study has shown increased macular thickness specific to MCI patients (Ascaso et al., 2014).

People with DS have a very high prevalence of developing early-onset AD (see section 1.3; Coppus et al., 2006; Holland, Hon, Huppert, Stevens, & Watson, 1998; Visser, Aldenkamp, Van Huffelen, & Kuilman, 1997), with almost 100% showing the neuropathological features by the age of 50 years (Head et al., 2007; Mann & Esiri, 1989; Wisniewski et al., 1984). It can be argued that the pathological features of AD are an inevitable part of ageing in people with DS. Studies which have looked at the DS retina using OCT include five studies which are covered in more detail in section 1.2.5. Two of these are case studies investigating specific ocular diseases in individuals, another two are OCT examinations of DS children and the final study investigates three participants with DS including 2 adults. This final study found thicker paramacular, significantly thicker fovea region and inner nuclear layer (INL) when comparing to non-DS controls (Laguna et al., 2013). This study also found that thicker inner layers of the retina were reported in trisomic *TsDyrk1a⁺⁺⁺* mice, removal of the third *Dyrk1a* gene decreased the thickness of the retina to normal values. *Dyrk1a* has an inhibitory effect of cell death and apoptosis during development (Laguna et al., 2008) which may account for altered cellularity. The two studies investigating the retina in children with DS

both found a thicker fovea in children with DS when compared to controls (O'Brien et al., 2015; Weiss et al., 2016), in addition O'Brien (2015) reported thicker areas in the outer retinal layers and macular. Weiss et al. (2016) reported that retinal structure was not significantly different in children with DS. Histological data has shown that general organisation of the retina is the same between DS and control groups (Laguna et al., 2013), although most studies in this area are limited to case studies of individuals with particular ocular diseases, and others have not commented on retinal structure (Ginsberg, Bofinger, & Roush, 1977; Sergovich, Madronich, Barr, Carr, & Langdon, 1963).

3.3 Aims

The overarching aim of this project is to determine whether the retina has the potential to provide an early biomarker site for changes relating to AD in people with DS. This study is the first known to provide cross-sectional analysis of retinal thickness using OCT in adults with DS. The particular aims of the study reported in this chapter were to:

- Assess the acceptability and feasibility of OCT as an imaging technique in adults with DS.
- Compare the thickness of the RNFL, macular and individual retinal layers in people with DS to an age-matched typically developing control group and an older typically developing comparison group.
- Evaluate age-related changes in retinal thickness in the DS group and compare this to age-related changes in the typically developing group.

3.4 Hypotheses

- Retinal measures will be thinner in the DS group compared to the age-matched control group and will be more similar to the older age comparison group.
- Age-related retinal thinning will be exacerbated in people with DS.
- Superior and inferior RNFL quadrants will show particular thinning effects in the DS group.

3.5 Methodology

Full details of the study population, OCT materials, scan procedure and data presentation can be found in Chapter Two, sections 2.5, 2.6 and 2.7.

3.5.1 Study design

This study was a cross-sectional design comparing retinal thicknesses of adults with DS over the age of 30 years to an age-matched control group and older-age comparison group. All participants with DS had the characteristic phenotype of DS. Trisomy-21 was confirmed from medical history or physical characteristics. All participants in this study were considered to have capacity to consent to research and informed consent was gained from all participants.

Associations between age and retinal thickness were investigated in the DS and control groups. Additional investigations in the DS group were conducted based on suggestions from previous research that those over the age of 40 years exhibit abnormal A β binding in the brain (Annus et al., 2016). Analyses were conducted between groups of DS individuals under 40 years, and over 40 years.

3.5.2 Retinal scan procedure

Full details of the equipment and procedure of the retinal scans are presented in section 2.6. All retinal examinations were completed on the Spectralis Heidelberg Retinal Angiography + OCT (Spectralis OCT; S3600). Retinal examinations for all participants were conducted by Dr Eduardo Normando at one of three locations;

- Douglas House, Department of Psychiatry, Cambridge University
- ICORG Unit, Western Eye Hospital, London
- Down's Syndrome Association Headquarters, Langdon Down Centre, Middlesex

All scans were completed in one visit and scan procedure lasted approximately 10-20 minutes for the DS participants. Average scan time for the control group was less than five

minutes. After the scans were finished the participant was thanked and allowed to leave, there were no after effects of the scan procedure.

Data analysis was undertaken using IBM SPSS Statistics software and Heidelberg Eye Explorer version 1.9.10.0, Heidelberg Engineering, Spectralis Viewing Module version 6.3.4.0 (Heyex). OCT examinations completed in this study included a RNFL scan, and a macular and posterior pole scan. Each of these retinal regions will be considered independently in the analysis of this chapter. The RNFL is divided into five sections, including the four quadrants, superior, inferior, nasal and temporal, and the global RNFL thickness. The RNFL and macular data are presented as an average of the values from both eyes, whilst the posterior pole data of the eye with the highest quality index scan is presented.

Macular and posterior pole scans are centred on the fovea. The macular scan provided thickness data of the fovea, and the inner and outer macular area. The inner and outer macular are sub-divided into quadrants as with the RNFL scan, analysis of the macular quadrants were completed if there were significant results in the overall average of the macular. The posterior pole thickness data included the macular areas but also a wider area, for this data, the retina was segmented into inner and outer layers, which were analysed along with thickness data of the whole retina. In cases where there were significant results within the averaged layers, these were further divided into individual layers. Inner layers consist of the nerve fibre layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL) and inner nuclear layer (INL). Outer layers consist of the individual outer plexiform layer (OPL), outer nuclear layer (ONL) and retinal pigment epithelium (RPE) layer. Full details of the retinal scans are shown in section 2.7.

3.5.3 Participant demographics

Fifty participants with DS were included in the data analysis of this study. There were 63 control participants in total, within this group, participants were sub-divided into those whose ages matched those of the DS group (between 18 and 56 years), forming the age-matched group, and those who were older than 57 years and who formed the older comparison group.

Adults with DS over the age of 18 years were approached to take part in this study, in total 60 participants consented to the study. Four participants were excluded from the study for

meeting at least one of the exclusion criterion and two participants withdrew consent before scanning. Four participants were excluded post-scanning for data quality below the threshold deemed acceptable by Heidelberg (<15 quality index rating; Huang et al., 2012). Control participants were age-and-sex balanced with the DS group. Thirty-seven participants were recruited within the age range of the DS cohort; one participant was excluded due to low quality data, leaving a total of 36 participants. A comparison group of typically developing adults older than the DS group were recruited to test for similarities in retinal thickness, owing to the accelerated ageing seen in DS. The oldest DS participant was 56 years therefore all control participants 57 years and older were entered into the older comparison group. This group consisted of 27 participants. Demographics of all participants are shown in Table 3.1.

	N	Males, females	Mean age (range)
DS group	50	28, 26	36.76 (18-56)
Control group	36	13, 24	37.27 (21-56)
Comparison group	27	7, 20	68.33 (57-91)
<i>Total recruited</i>	118	48, 70	Range 18-91 years

Table 3.1 Demographics of the study groups

3.6 Results

3.6.1 Tolerability of retinal examinations

3.6.1.1 Pilot study

One of the aims of this study was to establish whether OCT scanning technology is suitable for use in adults with DS. This was first assessed in a pilot study of nine participants who completed the full scanning procedure. Fixation problems led to a loss of one out of 18 eyes culminating in a 95% success rate which was acceptable to continue to the main study where tolerability was continually assessed. It was not deemed necessary to make any changes to the scanning protocol at this time.

3.6.1.2 Cross-sectional study

For the cross-sectional study an additional 51 participants with DS were recruited, 60 participants in total, after exclusions and withdrawals a total of 50 participants were retained across both the pilot and cross-sectional studies. Total loss of all data due to poor quality was 7.4%, which is considered acceptable. If a participant was able to complete one of the scans with acceptable quality they were retained in the study. Completion rates differed substantially between the types of scans applied (see Table 3.2). The RNFL scan had a higher success rate with 47 participants completing the right eye scan and 42 participants completing the left eye scan, only one participant was unable to complete the RNFL scan in either eye. The macular/posterior pole scan had a higher loss rate, 38 participants completed the right eye scan and 33 participants completed the left eye scan. In the control group one individual's data was excluded from both eyes and all scans due to poor quality.

	Total N	Scans completed (right eye, left eye)	Success rate (right eye, left eye)
RNFL data	50	47, 42	94%, 84%
Macular/posterior pole data	50	38, 33	76%, 66%

Table 3.2 Scans completed in the DS group.

Scan procedure in the DS participants took between five and 20 minutes. This was often considerably longer than the average five minutes taken for the control participants. In general, participants with DS did not find the scanning procedure uncomfortable or distressing, although some reported that it was difficult to keep looking at the light. Retinal scans were not undertaken in any specific order during testing and no preference was given as to which eye was scanned first. Almost all participants were able to complete the RNFL scans and more than 50% completed the macular and posterior pole scans. This indicates that OCT technology is acceptable and feasible in the DS, with the expectation that there will be higher loss and slower scan times in comparison to control participants. Tolerability seen in the pilot study was not replicated in the cross-sectional study. Future studies in this area should make reasonable adjustments and improvements to the scanning protocol, as discussed in the limitations, for DS participants in order to reduce the number of failed scans.

3.6.2 RNFL thickness

Global RNFL data was normally distributed; however outliers were identified in both the DS group and the control group. Outlier sensitivity analysis was performed which showed that there was no impact on the significance value with the removal of outliers, additionally there did not appear to be anything specific about those participants identifying them as candidates for removal, therefore outliers were retained.

There was a statistically significant difference in the thickness of the global RNFL measure between groups as determined by one-way ANOVA ($F(2,111) = 47.46, p < .001$). Post-hoc analyses showed that the global RNFL was significantly thicker in the DS group compared to the age-matched control group ($p < .001$) and compared to the older comparison group ($p < .001$). There were no significant differences between the thickness measures of the age-matched control group and the older comparison group ($p = .407$). Means, standard deviations (SD) and ranges for all groups are shown in Table 3.3.

Study group	<i>N</i>	Mean \pm SD (μm)	Minimum, maximum values (μm)
DS group	47	116.44 \pm 12.7	77, 143
Age-matched control group	36	98.44 \pm 7.58	81.5, 122
Older comparison group	31	94.81 \pm 10.08	80, 120

Table 3.3 Descriptive data of the global RNFL thickness measures.

3.6.2.1 Axial length

Axial length was assessed in our population of participants with DS as it has been shown that shorter and longer eyes can alter the thickness measurement of the OCT scan (Kaliner et al., 2007). It was found that three of the participants had short eyes, two had long and one had extra-long. Global RNFL thickness measures were identified for each of these participants individually to see if excessive thin or thick measure were reported. None of the participants identified expressed measures considered to be outliers, nor were these measures at the range limits of the DS global RNFL mean (77-143 micrometres (μm)) therefore there was no indication that axial length impacted the data in this study.

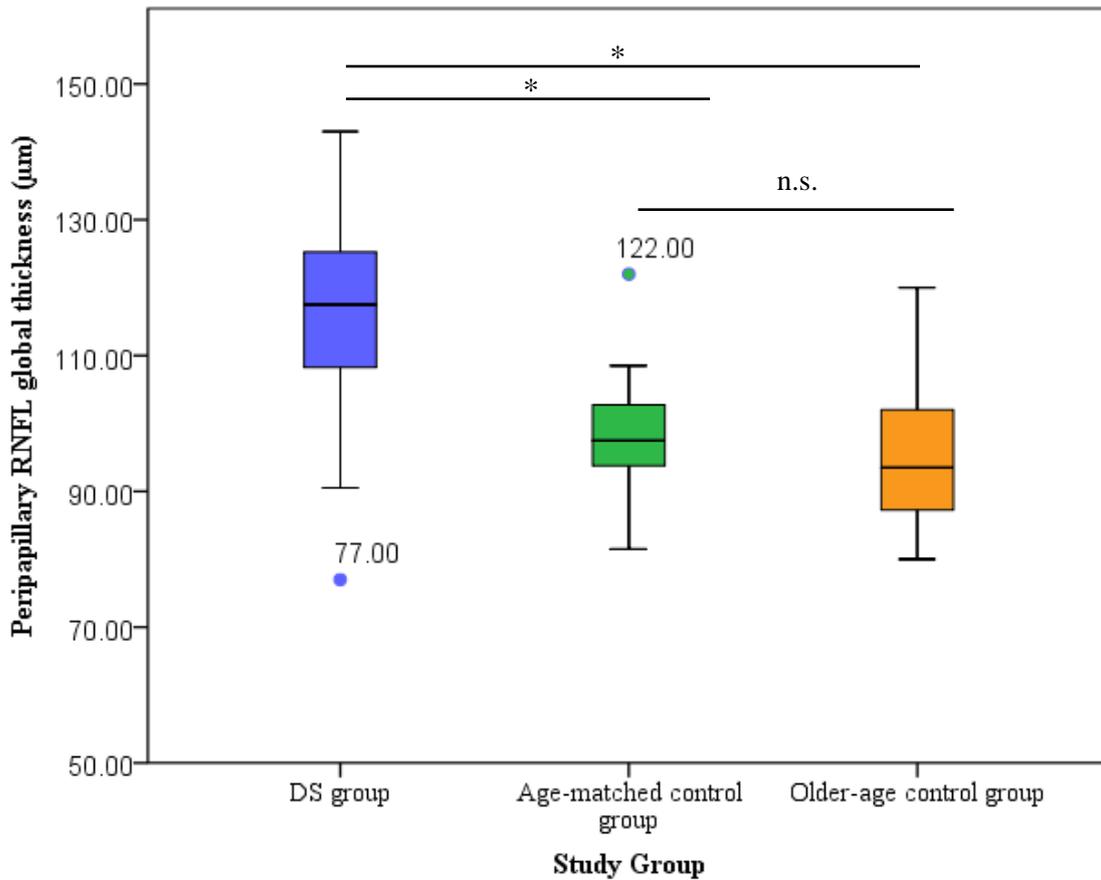


Figure 3.1 Box-plot of the spread of global RNFL thickness values between groups. Data shows that there are significantly thicker measures in the DS group compared to the control group and the older comparison group.

Figure 3.1 shows a relatively even distribution of values within groups, with similarly low measures between the two control groups and considerably higher thickness values in the DS group. The finding of significantly thicker global RNFL measures in the DS group compared to both the control group and the older comparison group is contrary to predictions. It was hypothesised that the DS group would show thinner measures than the control group and would have retinal thickness values that were statistically similar to the older comparison group. The older control group showed slightly thinner global RNFL than the age-matched control group, as expected.

3.6.2.2 *Peripapillary quadrant analysis*

The unexpected results of the global RNFL thickness have indicated that the peripapillary is thicker in DS, further analysis of the RNFL quadrants is required to determine whether this thickness is evenly represented across the quadrants or whether there is heterogeneity between quadrants. It was originally hypothesised that the superior quadrant would show increased thinning in the DS group in comparison to other quadrants.

Normality tests showed that data was normally distributed for all quadrants in both groups, except for the temporal quadrant in the DS group. One participant was identified as an extreme outlier, this individual had a measure of 151 μ m in the temporal quadrant where the range of the rest of the DS group was between 65 and 125 μ m (mean 93 μ m). This participant was female, 40 years and showed no evidence of dementia or low cognitive performance in areas associated with dementia. Due to the extremity of this result this case was removed from analysis.

Within-group quadrant analysis

Within group repeated measures analysis of the quadrants identified that in the DS group there were significant differences between the thickness measures of the quadrants ($F(2.66, 119.84) = 330.29, p < .001$). Mauchly's tests for Sphericity was assumed ($\chi^2(5) = 7.69, p = .174$) however the Greenhouse-Geisser correction was applied for caution. Further comparisons between quadrants were conducted using student t-tests which identified that all quadrants were statistically significantly different from one another in the DS group (all quadrants to all quadrants, $p < .001$).

In the control group there was also a significant difference between the quadrants as determined by within groups repeated measures analysis ($F(2.08, 72.89) = 193.41, p < .001$) with the Greenhouse-Geisser correction applied as Mauchly's test of sphericity was violated ($\chi^2(5) = 21.56, p = .001$). Further comparisons using student t-tests between the individual quadrants showed that there were no significant differences between the temporal and nasal quadrants ($p < .410$) or between the superior and inferior quadrants ($p < .055$). However nasal and temporal quadrants were both significantly thinner than both the superior and inferior quadrants ($p < .001$). Interpretation of the quadrant measure means indicated that the quadrants were not of homogenous thickness in either group. In the control group

thinnest to thickest ranking was as follows; temporal, nasal, superior, and inferior. This ranking was identical in the DS group apart from that the nasal and temporal quadrants were reversed.

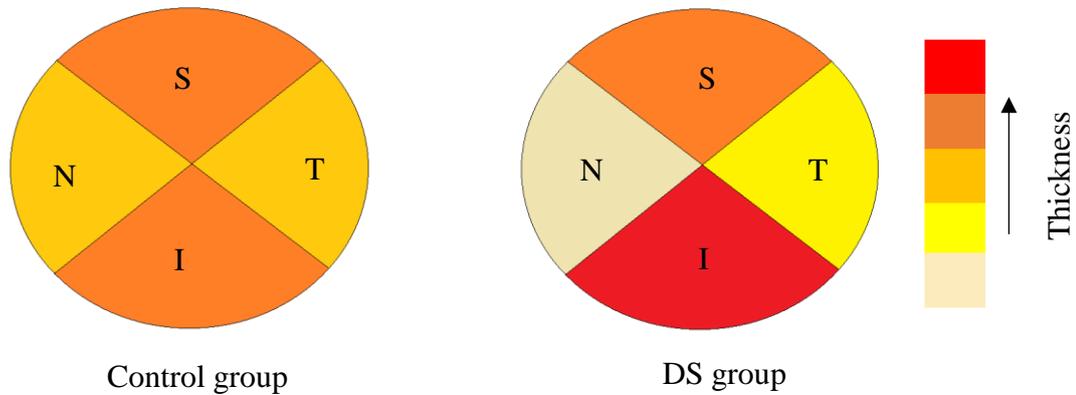


Figure 3.2 Schematic showing thickness order within the RNFL quadrants between groups. Same colours indicate no statistical differences within groups between the thickness values whilst different colours indicate statistically significant differences. Warmer colours represent thicker areas.

Between-groups quadrant analysis

Multivariate ANOVA statistics were conducted to determine statistical differences within quadrants between the two groups. There was a statistically significant difference in the quadrant thickness based on study group ($F(2)= 77.00, p < .001$). Univariate tests identified that there were statistically significant differences between the temporal ($p < .001$), superior ($p < .001$) and inferior ($p < .001$) quadrants of the RNFL between DS and the age-matched control group. The nasal quadrant was not statistically significantly different between groups ($p = .915$).

Large effect sizes ($< .50$; Cohen, 1992) were reported in the temporal, superior and inferior quadrants, this indicates that the effect accounts for 25% of the variance. A low effect size ($r = .01$) was reported in the nasal quadrant.

RNFL Quadrant	Mean (μ) \pm SD		<i>P</i>
	DS group (N=46)	Control group (N=36)	
Nasal	74.93 \pm 14.89	74.59 \pm 13.24	.952
Temporal	93.00 \pm 13.05	71.31 \pm 12.61	< .001*
Superior	142.01 \pm 19.04	121.40 \pm 12.15	< .001*
Inferior	154.70 \pm 22.7	126.33 \pm 15.26	< .001*

Table 3.4 RNFL quadrant differences between groups. *Results significant to the Bonferroni adjusted p-value.

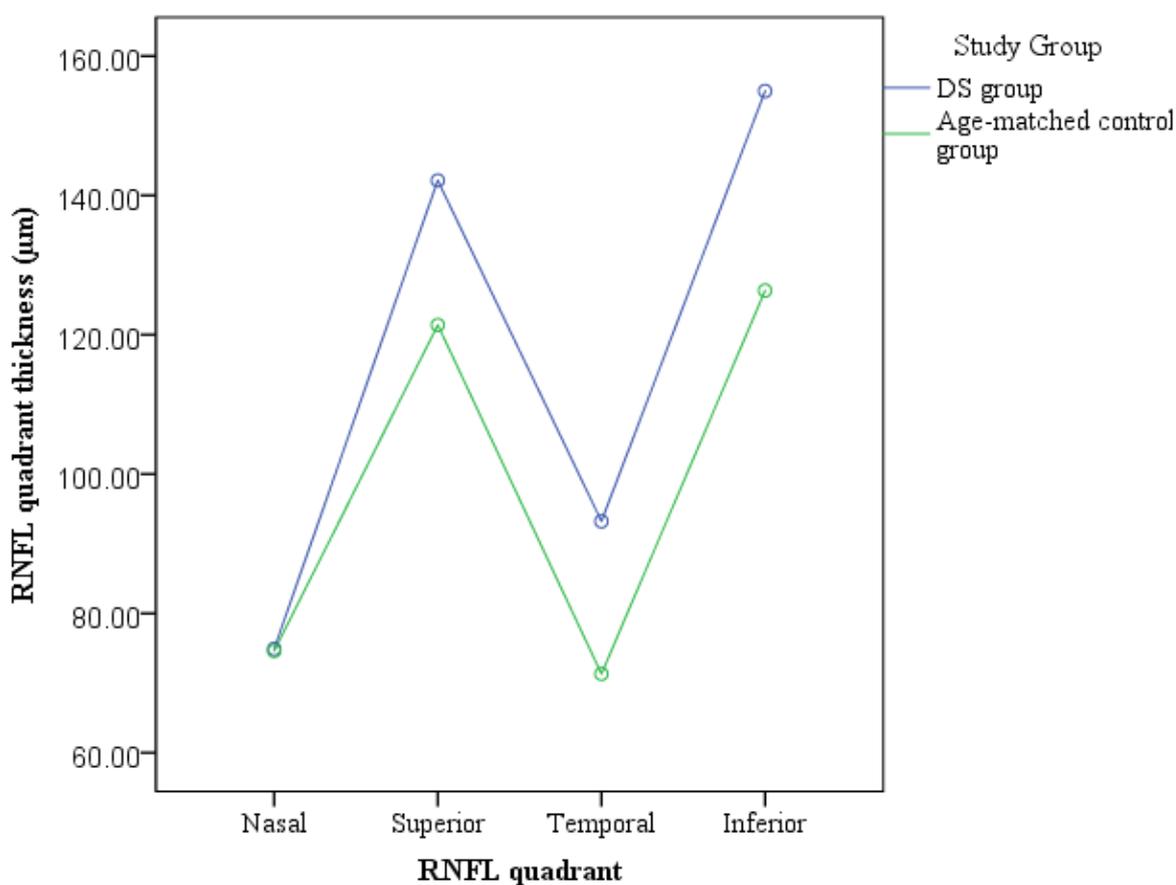


Figure 3.3 Line chart showing RNFL quadrant measures across groups. Apart from the nasal quadrant all quadrants were significantly thicker in the DS group.

These results show that the superior, inferior and temporal quadrants of the RNFL are significantly thicker in people with DS in comparison to age-matched controls; only the nasal quadrant is not significantly thicker in people with DS.

3.6.2.3 RNFL correlations with age

Within groups correlations were conducted to examine age-related changes in the retina between DS and control participants. We anticipated that the control group would show a trend towards thinning retina alongside increased age. Since the most RNFL loss is seen in typically developing people over the age of 50 years it was expected that the relationship would not be strong enough in the age-matched control group age range (18-56 years) to show significant loss. For the DS group, we expected to see a larger decline in RNFL loss with age that would start at a younger age and be more similar to the slope of decline seen in the older comparison group.

	DS group (N= 46)		Age-matched control group (N= 36)	
	<i>R</i>	<i>p</i>	<i>r</i>	<i>P</i>
Global (average of all quadrants)	-.041	.786	-.246	.148
Temporal average	.083	.584	-.163	.341
Superior average	-.079	.601	-.027	.877
Nasal average	.001	.998	-.128	.455
Inferior average	-.056	.714	-.238	.162

Table 3.5 Correlation statistics of age and RNFL quadrant

In the control group, there were small negative non-significant correlations between the RNFL measures and age, and a negative correlation between the inferior quadrant and age. Although there were no significant correlations for the age-matched control group there appeared to be a trend towards thinning in all regions, as was expected. In the DS group there were no significant correlations or trends between age and RNFL measure. This is contrary to our predictions that people with DS would show accelerated ageing in retinal thickness.

All controls (N = 67)		
RNFL region	<i>r</i>	<i>P</i>
Global average of all quadrants	-.262	.032
Temporal average	.247	.044
Superior average	-.673	<.001*
Nasal average	.479	<.001*
Inferior average	-.681	<.001*

Table 3.6 Correlations between RNFL quadrant thickness and age in the combined control group. *Results significant to the Bonferroni adjusted p-value.

Significant age-related correlations were identified when the older comparison group was included with the age-matched control data. This combination of groups gave a larger sample of 67 participants with an age range of 21-91 years. Large significant negative correlations ($r = -.681$ ($p < .001$) and $r = -.673$ ($p < .001$)) were seen in the inferior and superior quadrants respectively and a significant positive correlation of $r = .470$ ($p < .001$) was seen in the nasal quadrant. This data suggests that age-related thinning is seen in the superior and inferior quadrants but increased thickness was seen in the nasal quadrant. Evidence of a thinning retina, principally in the superior and inferior quadrants are supportive of previous literature in this area. The temporal quadrant and the global measure both showed small correlations that were not significant to the Bonferroni correction ($p > .01$).

Fisher's r - z transformation was calculated for comparison between the DS and full control sample correlation values. This statistic would determine whether there was a statistically significant difference between the two groups' correlations. Results showed that there were significant differences between the correlations of the DS group and the combined control groups for the superior, inferior and nasal quadrants ($p \leq .01$). This implies that the lack of age-related decline seen in the RNFL of the DS group is significantly different to age-related changes seen in typically developing adults. There were no significant differences between the correlations of the DS and age-matched control group only.

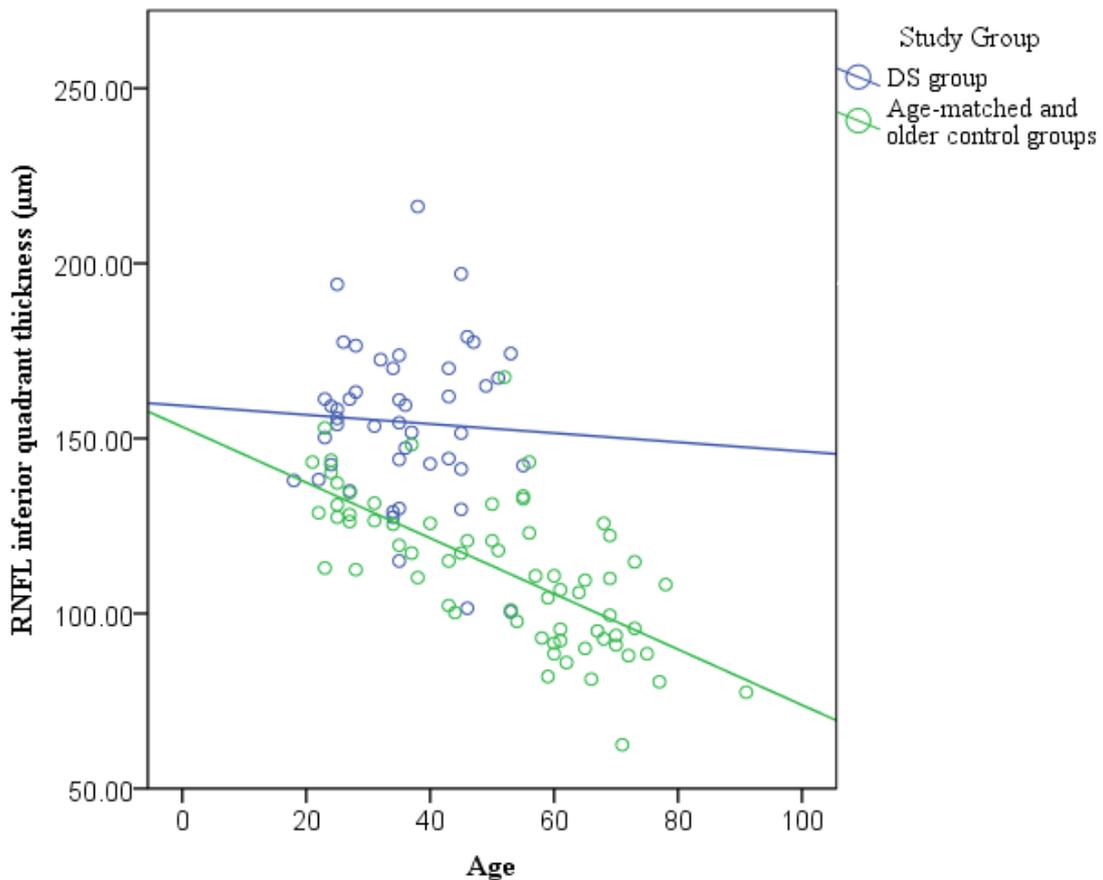


Figure 3.4 Scatterplot showing age-related differences between groups for the RNFL inferior quadrant.

3.6.2.4 Comparisons between DS age groups

Findings from our collaborative brain imaging study have shown a marked change in A β binding in adults with DS after the age of 39 years (Annus et al., 2016). With this in mind, further age-related changes were investigated by dividing the participants into two age groups, older DS (40–56 years) and younger DS (18–39 years). Retinal thickness of these groups were compared using student t-tests. It was predicted that the older DS group would show more evidence of thinning RNFL than the younger DS group.

Comparisons showed that there were no significant differences in the RNFL global measures ($t_{(39)} = .876, p = .386$), or the quadrant measures between the older and younger groups. This finding reinstates that no age-related differences were found in the DS group and that the expected age-related decline seen in the typically developing population is not seen in DS. The boxplot shows a larger spread of data in the older age group (see Figure 3.5).

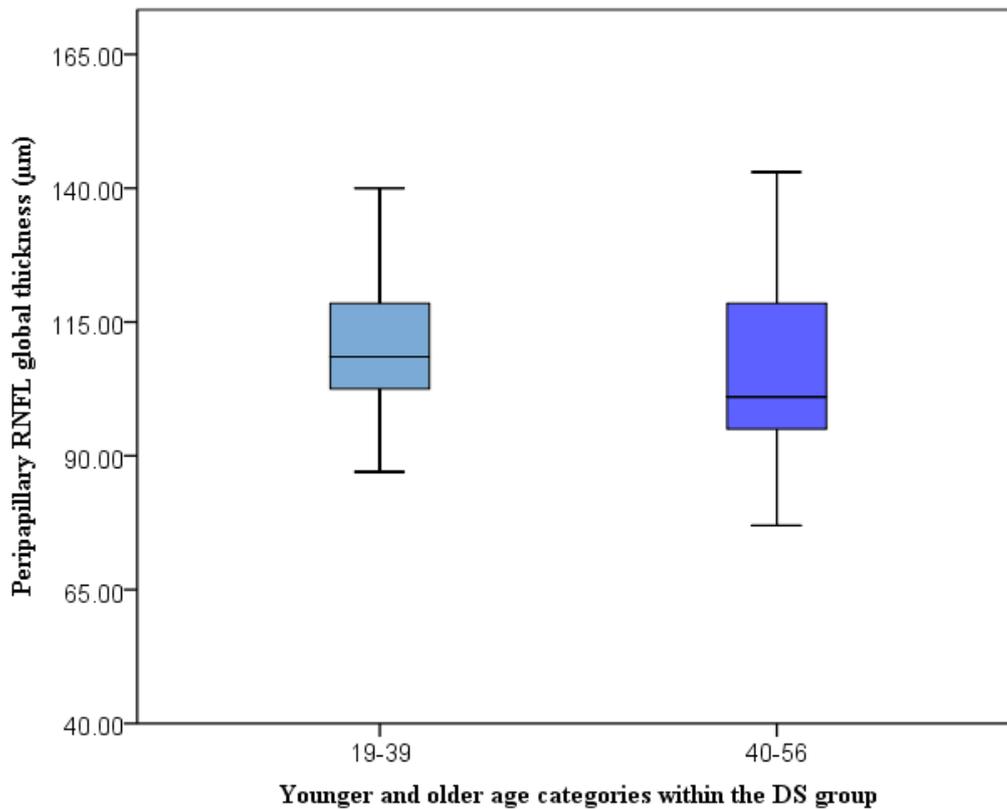


Figure 3.5 Boxplot showing no significant differences in RNFL thickness between the older and younger DS participants.

KEY FINDINGS: The key finding from the RNFL data analysis is that the global RNFL and the superior, inferior and temporal quadrants are significantly thicker in the DS group, which is opposite to hypotheses. In addition, age-related retinal thinning was not identified in the cross-sectional sample of DS participants as was seen in the control group (combined age-matched and older groups).

3.6.3 Posterior pole thickness

3.6.3.1 Averaged retinal layer comparisons

Analysis for the posterior pole data was completed using the eye with the highest quality index per participant. The averaged values for the overall retinal thickness and averages of the inner and outer layers are presented in Table 3.7. Data was normally distributed and there were no outliers.

	Mean \pm SD		<i>P</i>
	DS group (39)	Age-matched control group (37)	
Total retinal thickness average	313.23 \pm 15.49	299.5 \pm 13.09	< .001*
Inner layers thickness average	236.40 \pm 14.72	222.15 \pm 12.28	< .001*
Outer layers thickness average	77.25 \pm 1.43	78.19 \pm 2.03	.014

Table 3.7 Posterior pole thickness measures. *Results significant to the Bonferroni adjusted p-value.

One-way ANOVA results showed that there were significant differences between the groups for the average of all retinal layers ($F(1, 75) = 17.352, p < .001$) and for the average of the inner layers ($F(1, 75) = 20.14, p < .001$). The DS group had significantly thicker mean values for total and inner retinal layers, whilst for the outer layers there was no difference between groups. Medium effect sizes were calculated for the total ($r = .43$) and inner ($r = .46$) layers, outer layers showed a small effect size of $r = .28$. To determine in more detail the structural arrangement of thickness within the DS retina, the retinal layers were independently analysed.

3.6.3.2 Individual retinal layers comparisons

One-way ANOVA showed statistically significant differences in three of the four inner retinal layers and two of the three outer layers (Table 3.8). As with previous results, the DS group displayed significantly thicker retina in the majority of measures. Within the inner layers, only the NFL layer was not significantly thicker in the DS group. In the outer layers the RPE layer was not significantly thicker and the ONL was significantly thinner in the DS group ($F(1, 75) = 16.55, p < .001$). Whilst the finding of thinner ONL supports our original hypothesis, this results goes against the findings seen so far in this study.

Retinal layer	Mean \pm SD		<i>p</i>
	DS group (n=39)	Age-matched control group (n=37)	
<i>Inner layers</i>			
NFL	40.85 \pm 6.99	41.33 \pm 4.23	.722
GCL	40.89 \pm 3.94	33.42 \pm 2.40	<.001*
IPL	34.58 \pm 2.73	27.66 \pm 1.95	<.001*
INL	40.63 \pm 3.06	32.11 \pm 2.59	<.001*
<i>Outer layers</i>			
OPL	28.15 \pm 3.20	26.08 \pm 1.87	.001*
ONL	51.14 \pm 7.65	59.55 \pm 10.26	<.001*
RPE	13.20 \pm 1.01	12.84 \pm 0.93	.118

Table 3.8 Individual retinal layer thickness measures between groups. *Results significant to the Bonferroni adjusted p-value.

The ONL is the only region which has shown thinner values in people with DS, it was identified that that there was one outlier in each of the groups within this layer; this was further investigated in case the outliers were driving this reverse result.

The outlier in the DS group had much thicker ONL than the other participants, and the control participant thinner ONL, suggesting that removal of these outliers would only serve to heighten the difference between the groups. Nevertheless, to check the impact of these outliers, outlier sensitivity analysis was completed. With the removal of the outliers there was no change in the result of the independent t-test, still showing significantly thinner ONL in the DS group ($p < .001$) with a mean difference between groups of 10.58 μ m. Outliers were retained for the final analyses. The effect size between groups for the ONL was $r = .427$ which is a medium effect size supporting that there is a real difference between the two groups.

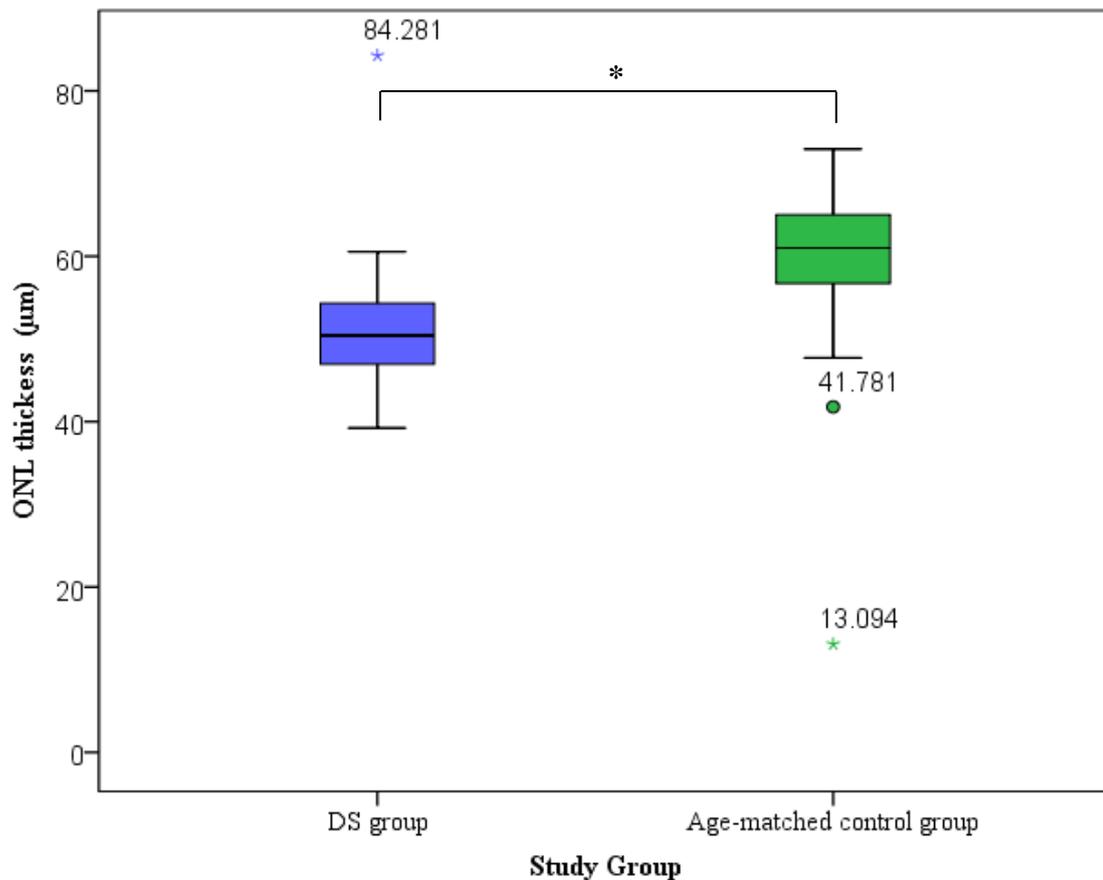


Figure 3.6 Outer nuclear layer thickness differences between groups.

3.6.3.3 Retinal layer correlations with age

Posterior pole thickness of all individual layers was correlated with age for both groups. It was predicted that the layers most likely to show an association with ageing in the DS group would be those that were not statistically thicker than the control group (NFL, ONL and RPE).

Results of these correlations in the DS group found no significant correlations between any of the retinal layers and age. Of the three retinal layers that were not significantly thicker in the DS group, there were no significant correlations (Table 3.9). The ONL showed a trend towards a positive correlation with age, which is contrary to the hypothesis that the DS retina will show thinning with age and controversial in that the ONL was the only area of the retina to be significantly thinner in the DS group.

No significant correlations with age were found in the control group. Trends towards negative correlations were seen in the GCL ($p = .012$), and the IPL ($p = .033$). All retinal layer correlations with age for both groups can be seen in appendix O.

Retinal layer	DS group (n=38)		Control group (n=37)	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>P</i>
NFL	-.087	.596	-.305	.066
RPE	.160	.329	.311	.061
ONL	.338	.035	-.236	.159

Table 3.9 Correlations between age and retinal layers. Retinal layers included are those which were not significantly different in thickness between the DS and the control group.

Using Fisher's r-to-z transformation the differences between the two correlation coefficients were assessed between the groups. There were no significant differences between the coefficients for the RNFL ($p = .342$) or the RPE ($p = .502$), however the ONL ($p < .0126$) differed significantly between groups indicating that age-related thickening of the ONL may be exclusive to the DS group.

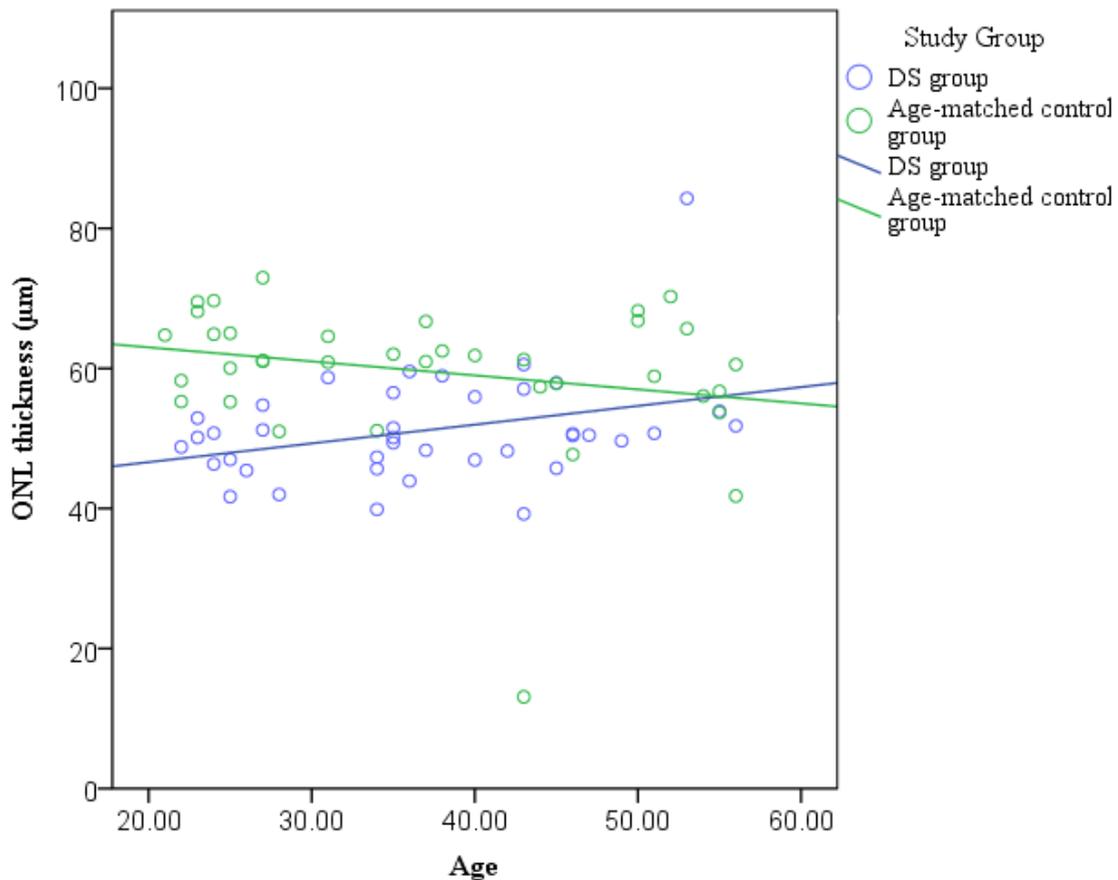


Figure 3.7 Age-related trajectory in ONL thickness.

The scatterplot in Figure 3.7 shows differences in cross-sectional age-related ONL thicknesses between the two groups. In the DS group there is a non-significant positive relationship with age ($r = .338$, $p = .035$) indicating increased thickness with age, whilst in the control group there is a non-significant negative correlation with age ($r = -.236$, $p = .159$). The difference between the two correlations is significantly different ($p < .0126$).

KEY FINDINGS: The key finding from the posterior pole region analysis is that the ONL is significantly thinner in the DS group compared to the age-matched control group. Although this result is supportive of the hypothesis, it is also opposite to all other retinal thickness analyses in this study. In addition, contrary to predictions, the ONL shows increased thickening with age, all other retinal areas showed no correlation.

3.6.4 Macular thickness

In the DS group, macular thickness data was not normally distributed in the fovea; non-parametric statistics are therefore applied as appropriate. The hypothesis for the macular region was that the DS group would show thinner average macular retina measures than the age-matched control group. Demographics of the macular retina thickness findings are presented in Table 3.10.

Macular region	Mean \pm SD (μm)		<i>p</i>
	DS group (N=37)	Age matched control group (N=36)	
Fovea	302.39 \pm 26.64	274.72 \pm 18.56	< .001*
Inner macular	355.15 \pm 19.81	345.95 \pm 13.47	.026
Outer macular	322.68 \pm 13.44	303.49 \pm 13.44	< .001*

Table 3.10 Macular thickness comparisons between groups. *Results significant to the Bonferroni adjusted p-value.

One-way ANOVA showed a significant difference between the DS and control groups ($F(1, 76) = 12.148, p < .001$). Between subjects effects showed significant differences in the fovea ($p < .001$) and the outer ring ($p = < .001$) but no differences in the inner ring ($p = .026$), see Figure 3.8.

The fovea and outer macular are significantly thicker in participants with DS, the inner macular is thicker in the DS group but this difference was not statistically significant. Figure 3.8 shows that the fovea has much lower values and smaller range in the control group. Figure 3.9 demonstrates the comparison between an individual with DS and an age-and-sex matched control participant, warmer colours indicate thicker values.

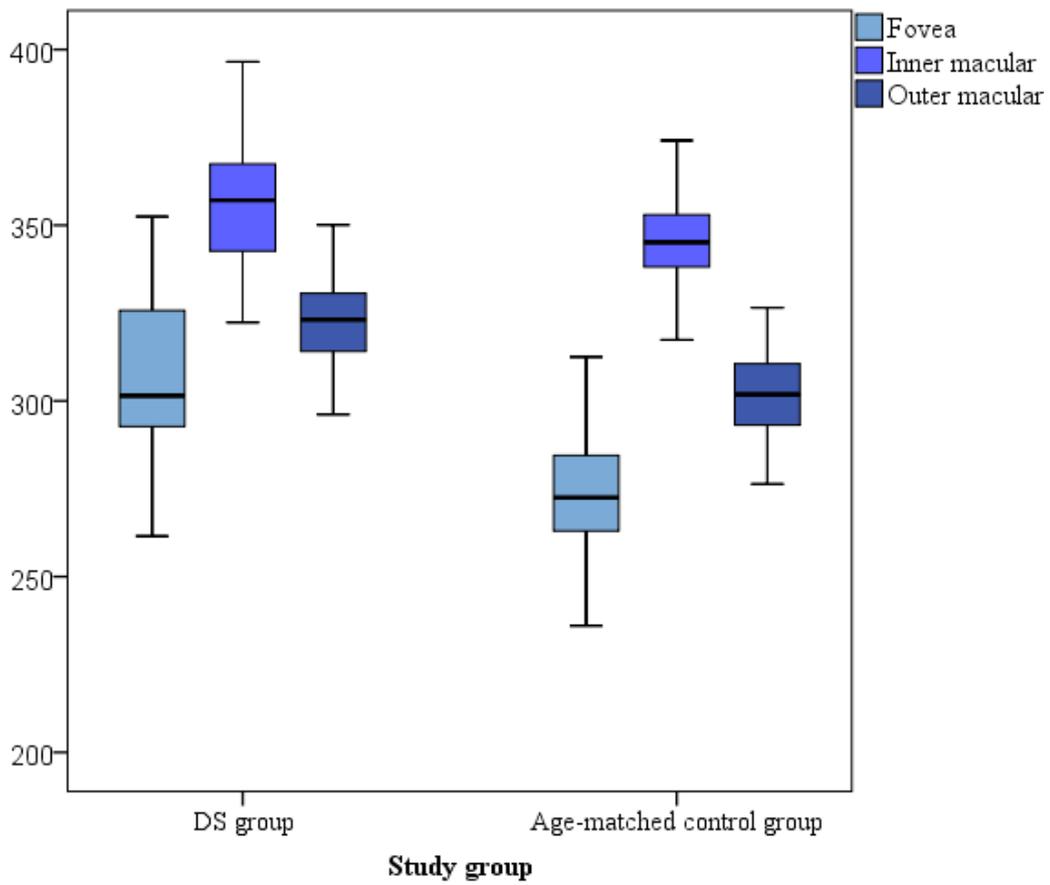


Figure 3.8 Box plot showing differences in macular thickness between groups.

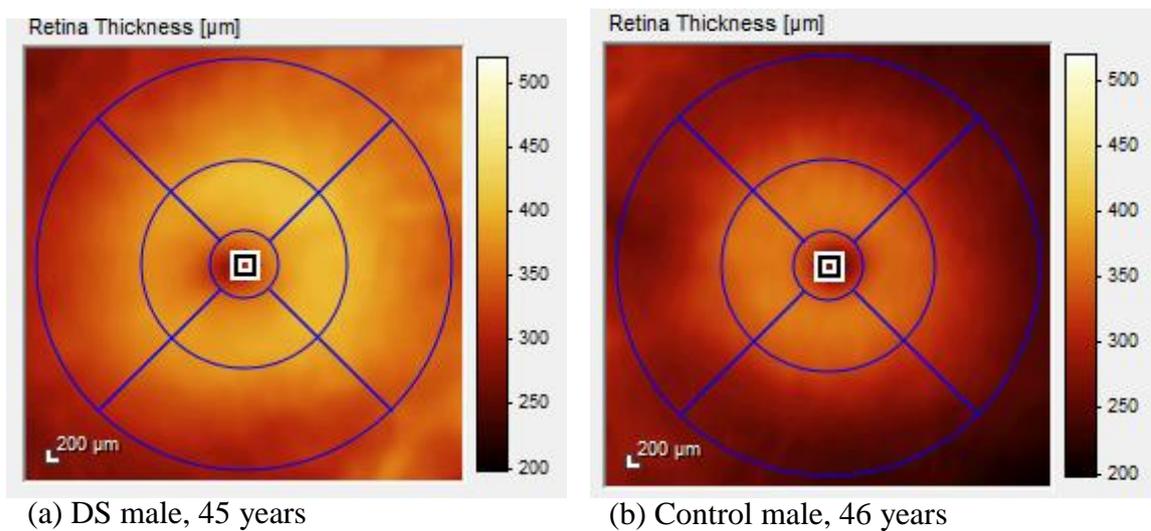


Figure 3.9 Heyex produced thickness colour maps between (a) DS participant and, (b) age-and-sex matched control participant.

3.6.4.1 *Quadrant comparisons between groups for the inner and outer macular regions*

Quadrant analysis within the macular rings was conducted to investigate homogeneity of thickness across the macular using paired samples t-tests. Normality tests showed that only the DS group superior quadrant of the inner macular was not normally distributed, no outliers were established. Non-parametric tests were used when analysing this quadrant.

Region	Mean \pm SD		<i>P</i>
	DS group (N=37)	Age-matched control group (N=37)	
<i>Inner macular</i>			
Temporal	342.36 \pm 21.10	334.84 \pm 13.37	.067
Superior	360.62 \pm 23.04	352.24 \pm 14.75	.017
Nasal	359.21 \pm 23.20	352.43 \pm 15.14	.135
Inferior	359.21 \pm 24.33	346.30 \pm 14.27	.343
<i>Outer macular</i>			
Temporal	307.69 \pm 16.64	292.57 \pm 13.15	< .001*
Superior	322.87 \pm 19.09	306.65 \pm 13.61	< .001*
Nasal	335.85 \pm 20.88	322.22 \pm 16.43	.002*
Inferior	316.87 \pm 16.19	296.54 \pm 15.47	< .001*

Table 3.11 Descriptive data for the inner and outer macular retina quadrants. *Results significant to the Bonferroni adjusted p-value.

Results of the independent student t-tests between groups are shown in Table 3.11. These results found that in the inner macular region all quadrants were thicker in the DS group than the age-matched control groups; however none were significantly thicker than the control group. For the outer macular all quadrants were significantly thicker in the DS group.

3.6.4.2 *Macular thickness correlations with age*

Age and thickness correlations were conducted for the fovea, inner and outer macular. As with previous retinal areas there were no significant correlations in the DS group (Table 3.12). In the age-matched control group there were no significant correlations, however all

correlations were negative. Macular quadrant correlations were not conducted as there was no evidence of age-related change in the DS macular.

Region	DS group (N=37)		Age-matched control group (N=36)	
	<i>r</i>	<i>p</i>	<i>R</i>	<i>p</i>
Fovea	.215	.189	-.189	.262
Inner macular	.023	.891	-.354	.031
Outer macular	-.067	.686	-.274	.101

Table 3.12 Correlations between macular regions and age.

Fisher’s r-to-z transformation was used to assess the significance of the difference between the correlations for each macular region. There were no significant differences for any macular regions, indicating that age-related change in the macular is not statistically different between DS and age-matched controls. The inner macular correlation for the control group appears to be trending towards a significant negative correlation whilst in the DS group there was no trend. To test this, the older comparison group was added to the age-matched control group and correlational analysis with age conducted for the inner ring, a significant negative correlation of $-.437$ ($p < .001$) was found which did differ significantly to the DS correlation when Fishers r-to-z transformation was applied ($p = .01$).

KEY FINDINGS: Key findings in the macular region are that the fovea and outer macular are significantly thicker in the DS group. No correlations were seen with age in the DS group, indicating an absence of age-related decline which was seen in typically developing controls.

3.7 Summary of findings

The aims of this chapter were threefold; firstly, to establish that the OCT methodology is an acceptable and feasible research method for use in people with DS. There was significant variability in individual focus for the scans, the RNFL scan had a much higher success rate than the macular/posterior pole scan. This suggests that a marker determined from the RNFL scan would be easiest to achieve in this population, however there are potential changes that could be made to the scanning protocol that would improve the success rate of the macular/posterior pole scan in the DS group, further studies are needed in order to refine the OCT protocol in people with DS and potential changes are discussed in section 3.8.1 Overall there was a loss of less than 25% for the macular scan indicating that for the majority of individuals both scans were achievable.

Secondly, retinal thickness measures in the DS group were compared to age-matched controls and to an older comparison group. Hypotheses for these comparisons were that the DS group would show thinner retina than both control groups, and that the superior and inferior quadrants of the RNFL would be most affected, as has been found in non-DS AD research. Contrary to hypotheses the findings showed that instead of thinner retina, the DS group had significantly thicker retinal regions in almost all areas when compared to the age-matched controls and the older comparison group. The older comparison group were originally included as a group where the expectation was that the DS group would show similar retinal thickness, due to the accelerated ageing seen in DS. However, results have shown that both the age-matched and older control groups have significantly thinner retina in comparison to the DS group. These findings indicate that people with DS have thicker retina than typically developing people.

Finally, the association between retinal thickness and age was assessed. In the DS group we found no significant correlations in any of the retinal areas assessed other than the ONL, which showed a significant positive relationship with age. This finding is again contrary to our hypothesis and suggests that the retina is not affected by age, or shows increased thickening with age. In the control group we saw trends towards thinner retina with increasing age as was expected and is supportive of findings in the typically developing literature, furthermore, when the age range was expanded to include the older comparison group many of the trends became significant. The result of this analysis indicates that people

with DS may have an atypical presentation of ageing of the retina, which is either shown as thickening of some areas or by an absence of thinning.

3.8 Discussion

Retinal thinning has been seen in studies investigating AD, with emphasis on thinning in the superior and inferior quadrants (Cunha et al., 2016; Kesler et al., 2011; Liu et al., 2015; Lu et al., 2010; Moschos et al., 2012). Nasal and temporal quadrants have been less frequently shown to be thinner in AD (Bambo et al., 2015; Iseri et al., 2006). In this study the only quadrant that was not significantly thicker in the DS group was the nasal quadrant. Initially, this seemed to suggest that the nasal quadrant could be more susceptible to age-related thinning than the other quadrants, however correlational analysis found no associations between the nasal quadrant thickness and age. Likewise no correlations were found with age for any of the other RNFL areas in the DS group, whilst in the age-matched control group there were trends suggesting thinning with age, as was expected, and which were heightened when the older comparison group were included.

The macular region and retinal layers are less frequently researched than the RNFL in AD literature. Studies which have investigated these areas have typically found that the macular shows evidence of age-related decline and significant further thinning in those with AD and patients with mild cognitive impairment (MCI; Cunha et al., 2016; Giménez, Dudekova, Gómez, & Lajara, 2016; Liu, Ong, Hilal, Miin, & Wong, 2016; Moschos, Markopoulos, Chatziralli, Rouvas, & Moschos, 2012; Polo et al., 2017). A controversial study in this area found thicker macular measures in patients with MCI (Ascaso et al., 2014). MCI patients showed thicker retina than both controls and AD patients, this data implies that thickening of the macular region may be an early event in AD disease progression and, as the disease advances, thinning of the retina becomes evident. This study supports the findings of our study, in that thicker macular, rather than thinner, is seen. As this was seen only in MCI there is a suggestion that this presentation of pathology may be part of early-stage AD. Comments from Ascaso et al., (2014) suggested that thickening of the macular may be caused by swollen neurons. Inflammation has been identified as one of the earliest hallmarks of several neurodegenerative diseases including AD (Fujino et al., 2004). This is supportive of the finding that thicker macular was only found in the MCI patients.

In the DS population it is also possible that inflammation, as an early AD-related pathology, could be responsible for the thicker retinal regions seen. This may also be related to inflammation caused by AD pathology as it is expected that many of the older DS group would have evidence of AD pathology particularly amyloid-beta (A β) deposits. Adults with DS and AD have extensive brain inflammation (Head et al., 2007). In AD research, studies have shown that there are significantly thinner retinal layers in AD compared to age-matched controls. This has predominantly been shown in the NFL, GCL, IPL and the ONL (Garcia-Martin et al., 2016). In people with DS the only research on retinal layers has been conducted in children, these studies found some evidence of thicker retina (O'Brien et al., 2015; Weiss et al., 2016), although not to the scale of generalised thickening across the retina that has been seen in this study. Findings of thicker retina in children imply that this may be a developmental feature of people with DS, although increasing thickness with age is suggestive that age-related pathologies are impacting on retinal structure.

The findings of these results raise two important issues in evaluating retinal thickness in people with DS. The first is that adults with DS were shown to have almost universally thicker retina in comparison to age-matched control participants and the second being accelerated ageing seen in DS. Whilst increased thickness found in DS children has not been linked with poorer eyesight or any detrimental effects (O'Brien et al., 2015), it is important to understand the underlying pathology that is causing increased thickness. It is possible that thicker retina is characteristic of people with DS; however this theory does not explain why this study found increased thickness with age in the ONL, and absence of normal age-related thinning in any retinal area. Another potential reason for thicker macular in people with DS has been identified by Wang et al., (2014), who found that children with cataracts displayed thicker fovea than children without cataracts. People with DS have a high prevalence of cataracts both in adult and childhood which could explain thicker macular in this area. This study excluded adults with DS with severe cataracts but did not exclude those with mild cataracts; therefore foveal thickness may be inflated in those with mild cataracts. If indeed thickening of the fovea is a reflection of cataracts this would still not explain the many other thicker retinal areas seen in this study in adults with DS. O'Brien et al., commented that due to limitations in the software used they had been unable to provide individual retinal layer analysis therefore had this been achieved there may have been evidence of further areas showing atypical thickness.

Increased thickness or lack of thinning could be a mask for underlying pathology. People with DS have a life-long over production of A β (Ballard, Mobley, Hardy, Williams, & Corbett, 2016), which may cause increased thickness, both as a primary result of the deposition and build-up of plaques in the retina, but also through a secondary measure as A β is known to trigger other events such as increased inflammatory response, microglial activity and apoptosis. Increased A β has been reported in the brains of children with DS as young as two years (Mehta, Capone, Jewell, & Freedland, 2007; Mori et al., 2002; Teller et al., 1996). Lifelong overproduction of A β would suggest that adults would have more retinal amyloid than children. This suggests that there may be patterns of thickness in the retina associated with age with some areas being affected earlier in life. Retinal amyloid has been seen in typically developing patients with AD (Koronyo-Hamaoui et al., 2011) and Rafii et al., (2015) are the first group to have studied the presence of A β in the retinas of adults with DS. This study used an oral supplement of curcumin that naturally binds and stains A β which can then be imaged non-invasively. The results of this study found significantly increased retinal amyloid in comparison to age-matched control participants and suggested that retinal A β is present in adults with DS without presence of dementia. The distribution of A β in the retina appeared to cluster around blood vessels, this is particularly interesting as people with DS have an increased number of vessels across the margin of the optic nerve (Kim, Hwang, Kim, & Yu, 2002; Ljubic, Trajkovski, & Stankovic, 2011; Williams, McCormick, & Tischler, 1973), this feature has not been associated with vision loss, or any affect to the individual and does not require any management, but may play a role with the deposition of A β and further explain increased thickness around the optic nerve head (ONH). Findings from Rafii et al., (2016) support the concept that A β could be responsible for thickening of the retina either directly or non-directly.

The findings of this study suggest that the DS retina does not exhibit the normal signs of age-related degeneration. Based on comprehensive knowledge of accelerated ageing in people with DS and evidence of ageing in many other physical and mental aspects (Zigman, 2013), it is unlikely that people with DS are exempt from ageing effects in the retina. It is more likely that the DS retina is affected by ageing, but that these effects are not presented in the same manner as in the typically developing population. Future studies should look to correlate presence of A β and location of depositions with measures of retinal thickness. The potential effects of overproduction and deposition of A β in the retina are considered in more detail in the general discussion chapter.

3.8.1 Limitations

Improvements to the methodology of the study

This study was able to recruit a fairly large sample of people with DS. A control group balanced for age and sex were recruited, and the mean values of age and sex were not significantly different between the groups. The age range of 18-56 years is reasonably representative of the lifespan of people with DS. It is possible that a larger age range (including children and older people with DS) would have allowed for more significant age-related correlations to be seen. In addition, imaging the retina in children would have been beneficial in further understanding the developmental potentiality of thicker retina. Another limitation of the age range in people with DS is that inevitably there will be a sample bias in the people who volunteered to take part in the study. Older adults with DS are more likely to have dementia and may not have the capacity in terms of travel and support to take part in studies, thereby limiting our data sample.

Improvements to the scanning protocol for improved data acquisition

There were several improvements that could have been made to the study. Scan completion was variable in the DS group, with many people struggling to remain fixated throughout the examination. There are several reasons why people many may have struggled with fixation. Firstly, there were several distraction factors, although attempts were made to keep the room quiet and dark there were often people walking around and talking outside the room – particularly at the Western Eye Hospital site as there were other studies being conducted in other rooms. At both sites parents or carers were invited to sit in the room with the participant if they preferred to, whilst this was initially suggested in order to make the participant more relaxed, the parent or carer would often talk to the participant and distract them from the task. In future studies more effort would be made to keep the room quiet and it would be suggested that parents or carers would only be in the room if the participant was distressed. It would have been preferable to have conducted the scans in a sequential order, this would have allowed us to see whether first or second scans were better completed as there may have been either an effect of tiring or an effect of practice in the participants. In order to improve the quality of the data collected, pupil dilating drops would be applied in future studies, whilst dilation was not necessary for the collection of data in this study, the effects of slight movements can be reduced if the pupils are dilated.

Additional technology

Findings from this study have shown that there may be underlying pathology in people with DS that is leading to thicker retinal structure. Additional optical scanning methodologies would be useful in investigating this further. Since the start of this study A β has been visualised in people with DS using recent technology, NeuroVision. Rafii et al., (2015) have used this technology to successfully image A β in the retina, in this study the combination of A β analysis and retinal thickness would have allowed for pattern analysis for A β deposition and corresponding areas of retinal thickness.

3.9 Conclusion

The results of the analyses of this chapter have shown that there is a significantly thicker retina in the DS group when compared to age-matched controls. The retina in people with DS also appears to undergo atypical ageing. These findings were contrary to our predictions that people with DS would have thinner retina and show increased and earlier age-related thinning of the retina. These predictions were based on results in normal ageing and in AD patient groups, along with evidence of accelerated ageing and high prevalence of early onset AD in people with AD. Possible causes of increased retinal thickness in people with DS have been discussed, including developmental characteristics of DS, and AD-related pathology including A β and A β induced pathology. These theories are considered in more detail in the general discussion along with the findings of the following results chapters.

Chapter Four:

Associations between retinal thickness and assessments of cognitive decline and dementia

4.1 Chapter Introduction

The aim of this chapter is to examine the relationship between retinal thickness, cognition, and dementia in adults with Down's syndrome (DS). Cognitive assessments were completed using the Cambridge Cognition Examination (CAMCOG) and an executive function battery, onset of dementia was assessed using the Cambridge examination for mental disorders of older people with DS and others with intellectual disabilities (ID) informant interview (CAMDEX). In this chapter, analyses are undertaken to examine the associations between cognitive performance and retinal thickness, as well as to establish retinal thickness differences in DS individuals with and without a diagnosis of dementia.

4.2 Background

One of the dominant characteristics of DS is the presence of an ID; typically people with DS have a mild-moderate ID however there is substantial variation in the extent of disability between individuals. Abnormalities and slow development in learning, memory and language are characteristic (Lott & Dierssen, 2010). Spatial working memory, particularly in sequential spatial tasks (Lanfranchi, Carretti, Spanò, & Cornoldi, 2009) is impacted, as is verbal short term memory, and explicit long-term memory (Brown et al., 2003; Hodapp & Dykens, 2005). Executive function is poorer in people with DS (Kittler, Krinsky-McHale, & Devenny, 2006; Luria, 1966). Visuospatial short term memory and implicit long term memory has been found to be more often preserved (Lanfranchi, Baddeley, Gathercole, & Vianello, 2012; Laws, Glynis, Bishop, & Dorothy, 2003).

In sporadic Alzheimer's disease (AD) the earliest changes relating to onset of dementia are almost always memory-related, however in people DS the early signs are more akin to changes seen in "dementia of the frontal type" (DFT; Ball et al., 2006; Gregory & Hodges, 1993). It is hypothesised that as the frontal lobes are underdeveloped in DS (Pinter et al., 2001), neuropathological changes may have earlier effects on the cognitive functions

associated with this area (Holland, Hon, Huppert, & Stevens, 2000; Mortimer, 1988). In DS, the earliest dementia related changes seen are often in personality and behaviour, including withdrawal, apathy and stubbornness (Holland et al., 1998). Holland et al., (1998) reported that many individuals who failed to meet the criteria for AD met the behavioural criteria for DFT, and later progressed to a diagnosis of AD within five years (Ball et al., 2006). Diagnoses of DFT are higher in younger participants (<45 years). Cognitive tasks that are used in the assessment of dementia in DS have been traditionally devised for educational assessment, rather than neuropsychological assessment, therefore do not cover the full range of cognitive abilities known to decline in dementia, and are not always designed so that floor and ceiling effects can be avoided (Hon et al., 1999). The CAMCOG neuropsychological test aims to address these issues, and is used together with the CAMDEX informant interview (Roth et al., 2014). CAMCOG is a brief neuropsychological battery which has been extensively used to assess a range of cognitive functions including; language, praxis, memory, orientation, abstract thinking, attention and perception. All of these are items included in the Mini Mental State Examination (MMSE (Folstein et al., 1975), however they are tested more rigorously in the CAMCOG.

Due to ID and low IQ in people with DS, cognitive functioning is predictably decreased, and it is often difficult to disentangle developmental cognitive deficiency from changes that may be due to the onset of dementia. CAMCOG scores alone are not sufficient to confirm a diagnosis of dementia in people with DS. As discussed in section 1.3.3, assessing dementia in people with DS presents with a range of complications, some of which are due to the presence of ID (Aylward, Burt, & Thorpe, 1997), which has led to varied prevalence rates of dementia being reported (Coppus et al., 2006; Lai & Williams, 1989; Oliver & Holland, 1986; Sekijima et al., 1998; Tyrrell et al., 2001; Visser, Aldenkamp, Van Huffelen, & Kuilman, 1997). As presentation of dementia in people with DS is different to that of typically developing people, changes in behaviour and personality are often overlooked, memory changes are more readily noted, but these often occur later in the progression of AD in people with DS. Certainly by the time memory changes are seen, but also most probably at the time that changes in personality and behaviour are seen, it is very likely that AD neuropathology is already established in the brain (Bateman et al., 2012).

Generally, people with DS are not aware enough of their own capabilities and limitations to give an account of recent changes in their functioning levels; therefore, in the majority of cases, reliance is placed on the individual's friends, relatives and carers. This can be

problematic, often changes relating to dementia are gradual and those who spend a lot of time with the individual will have difficulties pinpointing the start of change, on the other hand, particularly in the case of those in residential care, high turnover of staff or low contact hours mean that changes can be overlooked and not efficiently reported (Holland & Walpert, 2016). Structured interviews, such as the CAMDEX, are used to identify changes. Interviews are completed with a person who has known the individual with DS well for a minimum of six months. These interviews are designed to establish differences between the individual's capabilities now, from a time before the changes started. This interview can also help exclude the pseudo-dementias (i.e. depression, hypothyroidism, psychosis) which often present with similar symptoms as dementia (Wells, 1979). For the most accurate testing, the best method would be to have CAMCOG and CAMDEX completed at a time where the person was "at their best" and not showing any signs of behaviour, personality or memory change. This would then be used as a baseline for changes in future assessments.

The MMSE (Folstein et al., 1975) is the most widely used screening instrument for detection of cognitive impairment, however there are many different cognitive tests available. In this study, the Telephone Interview for Cognitive Screening (TICS; Brandt, Spencer, & Folstein, 1988) was used for dementia screening of the control group participants. This test was developed with the primary intention of creating a screening assessment with the same impact as the MMSE, but which could be used over the phone. The TICS has a very high correlation with MMSE (Pearson's $r = .94$). The TICS has high test-retest reliability, high sensitivity and specificity for cognitive impairment in AD. The main advantage of the TICS is that there is a lower dropout rate as the burden on the participant is reduced. This is particularly relevant in people with dementia who may not be able to travel easily.

To date, no research has compared the cognitive status or onset of dementia with retinal thickness in people with DS. In sporadic AD, several studies have assessed the relationship between retinal thickness and cognitive status, most commonly using the MMSE. The findings of this research have mixed results, with some studies being unable to show any associations between cognitive scores and retinal thickness, whilst others which have found lower MMSE scores are correlated with thinner retinal measures. There is large variability in the strength of the associations seen across the literature. Moreno-Ramos, Benito-León, Villarejo, & Bermejo-Pareja, (2013) reported a high correlation between poorer cognition and thinner retina of $r = .96$, whilst (Cunha et al., 2017) reported a much lower value of $r = .33$. Some studies have recognised the inferior quadrant of the RNFL as being the most

associated with lower MMSE scores (Ascaso et al., 2014; Berisha et al., 2007). The inferior quadrant, along with the superior quadrant were also found by many studies to be the most likely to show thinning in the AD and mild cognitive impairment (MCI) patient groups (Eraslan et al., 2015; Liu, Duggan, Salt, & Cordeiro, 2011; La Morgia et al., 2015). Outside of the MMSE, other cognitive tests have been associated with the temporal quadrant of the RNFL (Knoll et al., 2016; Shen et al., 2014). In contrast to all other cognitive associations found, these studies reported that thicker retina was associated with poor cognitive function. More robustly, findings have shown differences between age-matched healthy controls and AD patients retinal thickness in the RNFL (e.g. Garcia-Martin et al., 2016; Larrosa et al., 2014; Marziani et al., 2013) and the macular (e.g. Cunha et al., 2016; Khawaja et al., 2016; Moschos et al., 2012; Polo et al., 2017) indicating that cognitive scores may not be sensitive enough to correlate with retinal thickness.

Due to the nature of ID in people with DS it is important to distinguish between cognitive ability as a developmental factor and cognitive performance, affected by onset of dementia. To accomplish this, retinal thickness measures were correlated with IQ (developmental cognitive ability) and cognitive tests that are specifically designed to assess areas known to decline with age and neurodegenerative disease.

4.3 Aims

- To assess the association between developmental cognitive ability (IQ) and retinal thickness in people with DS.
- To investigate the relationship between cognition as a measure of age-related decline (CAMCOG and executive function scores) and retinal thickness.
- To compare retinal thickness of individuals with DS and a diagnosis of dementia to DS without dementia as determined by the CAMDEX informant interview.

4.4 Hypotheses

Hypotheses have been formed based on literature from non-DS patients with sporadic or late-onset AD. In addition to the hypotheses stated, exploratory analysis was undertaken in the retinal areas that were highlighted in the previous results chapter.

- Cognitive performance will positively correlate with retinal thickness.
- The superior and inferior regions of the RNFL will correlate with cognitive scores.
- Participants identified as having a diagnosis of dementia or having significant functional or behavioural change will show thinner retinal measures than age-matched DS participants without dementia.

4.5 Methodology

Full details of the study population, neuropsychological test materials, retinal scan procedures and data presentation can be found in Chapter Two, sections 2.5, 2.7 and 2.8.

4.5.1 Procedure

Neuropsychological tests were conducted to provide a comprehensive cognitive assessment for the DS participants. These tests were conducted either at the testing site or the participants' homes over one or two visits depending on the participant's preference.

Full details of the neuropsychological tests are given in section 2.8. In summary, tests applied in the DS group included the CAMCOG and an executive function battery to assess areas known to experience decline in people with dementia. The Kaufman Brief Intelligence Test-2 (KBIT-2; Kaufman & Kaufman, 2004) was used to measure IQ. Neuropsychological testing lasted approximately one to two hours, this was often split over two sessions. For the control group, dementia screening assessments were completed using the TICS, this test takes around five minutes to complete and was either conducted in person or via telephone call.

Scoring and assessment of the neuropsychological tests were completed by the researcher. Non-standardised KBIT-2 scores were analysed rather than standardised scores. Standardised scores are influenced by age, giving a floor effect at 40 points. Non-standardised scores of the KBIT-2 were used in this analysis to remove the issue of floor effects in a population of adults with ID who typically show lower mental age.

Dementia interviews for the DS participants were completed with the individual's main parent or carer. In most cases, this was a parent. Interviews were completed at the

individual's home or testing site and lasted approximately 30-60 minutes. Assessment of the dementia interviews were completed by two consultant psychiatrists independently. Generally, participants with evidence of decline in two or more areas which did not meet the criteria for pseudo-dementias were given a diagnosis of dementia. Control participants were screened for dementia using the TICS assessment. This test was conducted by the researcher either at the testing site or over the phone, and lasted approximately five minutes.

4.5.2 Participant demographics

Fifty participants with DS completed at least one retinal scan, 46 of these participants also completed the CAMCOG and KBIT-2 assessments and 41 participants completed the executive function battery. Participants without a neuropsychological test score either refused to complete the tests, but still wished to remain in the study, or did not complete the battery, incomplete tests were not entered. Dementia interviews were completed for all participants. For the control group, all participants completed a short TICS dementia screening assessment.

In total, five participants were identified as having a diagnosis of dementia. The executive function battery was not completed by five of the DS group, including two of those with dementia. One of the DS participants (male, 53 years) was excluded from the analysis as the results of his CAMDEX interview were inconclusive. It was independently agreed by two clinical psychiatrists that this individual did not meet the criteria for dementia or pre-clinical dementia, but that he was exhibiting peculiar behaviours perhaps as a result of a pseudo-dementia. CAMCOG scores from this individual were found to remain stable over a period of five years (from involvement in previous Defeat Dementia in DS (DiDS) studies in our research group), supporting a diagnosis of no dementia.

4.6 Results

4.6.1 Neuropsychological test results

Table 4.1 shows the descriptive statistics of the DS and control group neuropsychological tests.

DS participants	N	Mean \pm SD (μ m)	Range
CAMCOG	46	76.96 \pm 19.52	17 – 105
KBIT-2 non-standardised score	46	58.57 \pm 21.08	7 - 102
Executive function score	41	39.80 \pm 9.42	13 – 53
Control participants			
TICS score	69	34.25 \pm 2.81	27 - 40

Table 4.1 Descriptive statistics of the neuropsychological tests

Large ranges were seen in all neuropsychological measures, however floor and ceiling effects were not observed which indicates that the measures are capturing true cognitive ability.

Individuals with DS with a diagnosis of dementia include two females aged 47 and 51 years and three males aged 49, 56 and 53 years. CAMCOG scores for these individuals ranged between 19 and 90, which were within the range of scores seen for the DS group without dementia (17-105). There were no significant differences in the scores on the CAMCOG battery between individuals with and without dementia ($t_{(44)} = 1.731$, $p = .091$), however scores were significantly different between the groups for the executive function battery, ($t_{(39)} = 2.156$, $p = .037$). In both cases the group without dementia had higher average scores than the group with dementia.

Correlations between age and the neuropsychological measures were conducted in the DS group in order to establish whether age was a confounding variable. Results showed that age was not correlated significantly with the CAMCOG score ($r = -.138$, $p = .354$), executive function battery ($r = -.271$, $p = .083$), or the non-standardised IQ scores ($r = -.126$, $p = .398$). CAMCOG and executive function scores were not normally distributed, which persisted after removal of the dementia group from the analysis, non-parametric tests are therefore used to analyse results. Non-standardised KBIT-2 scores were normally distributed.

For control participants, a score of 26 or lower in the TICS examination is an indication of mild impairment or, below 20, or moderately/severely impaired. All control participants scored at least 27 points indicating no dementia or mild impairment in the control group. It is possible that scores may have been reduced in this study due to, (a) the test being devised for use in the US, and, (b) language difficulties in some participants where English was not their first language, since all participants scored above the threshold this is not a limiting factor for this study but may be considered in future studies. Tests for normal distribution showed that the TICS scores for the control group were normally distributed.

4.6.2 IQ associations with retinal thickness

Non-standardised KBIT-scores were correlated with retinal thickness using Pearson's correlation. Non-significant correlations were reported in all retinal regions (Table 4.2).

	KBIT-2 Non-standardised scores	
	Correlation coefficient	P
Global RNFL	.171	.267
Total retinal layers average	-.083	.624
Fovea	.032	.851
Inner macular	-.042	.807
Outer macular	-.166	.334

Table 4.2 Correlations for non-standardised KBIT-2 scores and retinal thickness.

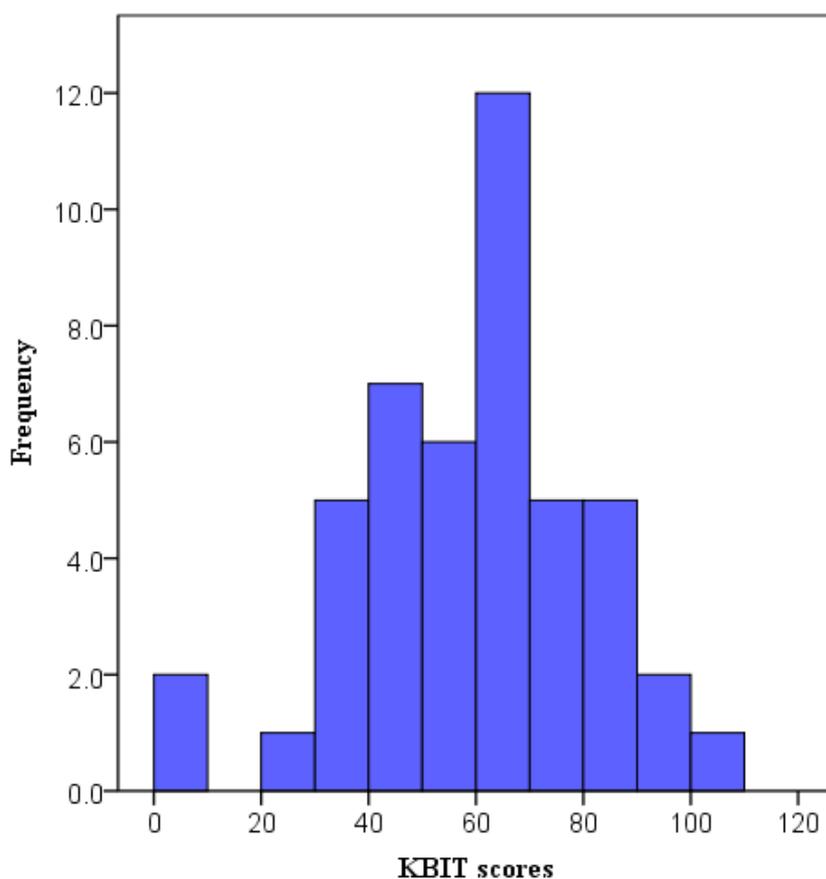


Figure 4.1 Histogram showing the non-standardised IQ scores for the DS group.

The histogram in Figure 4.1 shows the spread of the non-standardised scores (K-BIT scores) within the DS population. Results have shown no correlations with retinal thickness, which implies that developmental cognitive abilities are not related to retinal thickness and therefore the extent of an individual's ID is not a confounding variable in this study. Data shows a skew towards the upper end of the scoring, suggesting that the majority of participants included in this study had a mild/moderate ID rather than a severe/profound ID. The population sample may not be representative of all people with DS.

4.6.3 Retinal measure correlations with CAMCOG and executive function

It was predicted that RNFL thickness would positively correlate with CAMCOG and executive function scores. Furthermore, it was predicted that the superior and inferior quadrants of the RNFL would be implicated with cognitive measures, as has been shown in AD literature. Spearman's rho correlations were used to assess the association between the retinal measures and the CAMCOG and executive function battery.

4.6.3.1 Retinal nerve fibre layer analyses

No significant correlations were found between any of the RNFL measures and the CAMCOG or executive function battery. This study found no relationships with cognitive scores in any of the quadrants for the CAMCOG, the executive function battery showed a trend towards a small correlation with the superior quadrant.

	CAMCOG		Executive function battery	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
RNFL average	-.027	.867	.114	.461
Temporal	-.147	.367	-.186	.227
Superior	.217	.178	-.270	.076
Inferior	-.248	.122	-.003	.983
Nasal	.114	.484	.221	.149

Table 4.3 RNFL thickness measure correlations with CAMCOG and executive function battery scores

4.6.3.2 Macular analyses

For the fovea, inner macular and outer macular there were no significant correlations or trends identified between macular thickness and cognitive score. All correlations were between -.1 and +.1 indicating no correlations. Based on this finding, individual quadrants of the inner and outer macular quadrants were not assessed.

4.6.3.3 Retinal layers analyses

The average thickness of the total retinal layers, inner and outer layers was correlated with cognitive scores. Results showed that the outer layers showed a significant correlation with the CAMCOG scores. This result indicated that thinner outer layers correlated with lower CAMCOG scores, which supports our hypothesis (see Figure 4.2).

	CAMCOG		Executive function battery	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Total retina average	.140	.409	-.041	.817
Inner layers average	.074	.664	-.094	.599
Outer layers average	.423	.009*	.112	.529

Table 4.4 Average retinal layer thickness correlations with CAMCOG and executive function battery scores. *Results significant to the Bonferroni adjusted p-value.

Further investigation of the individual layers within the outer layers measure showed that no layer individually significantly correlated with CAMCOG scores. The outer plexiform layer (OPL) showed the closest trend to a significant correlation ($r = .312$, $p = .060$). The other layers; outer nuclear layer (ONL, $r = -.079$, $p = .644$) and RPE ($r = .199$, $p = .238$) did not show any trends.

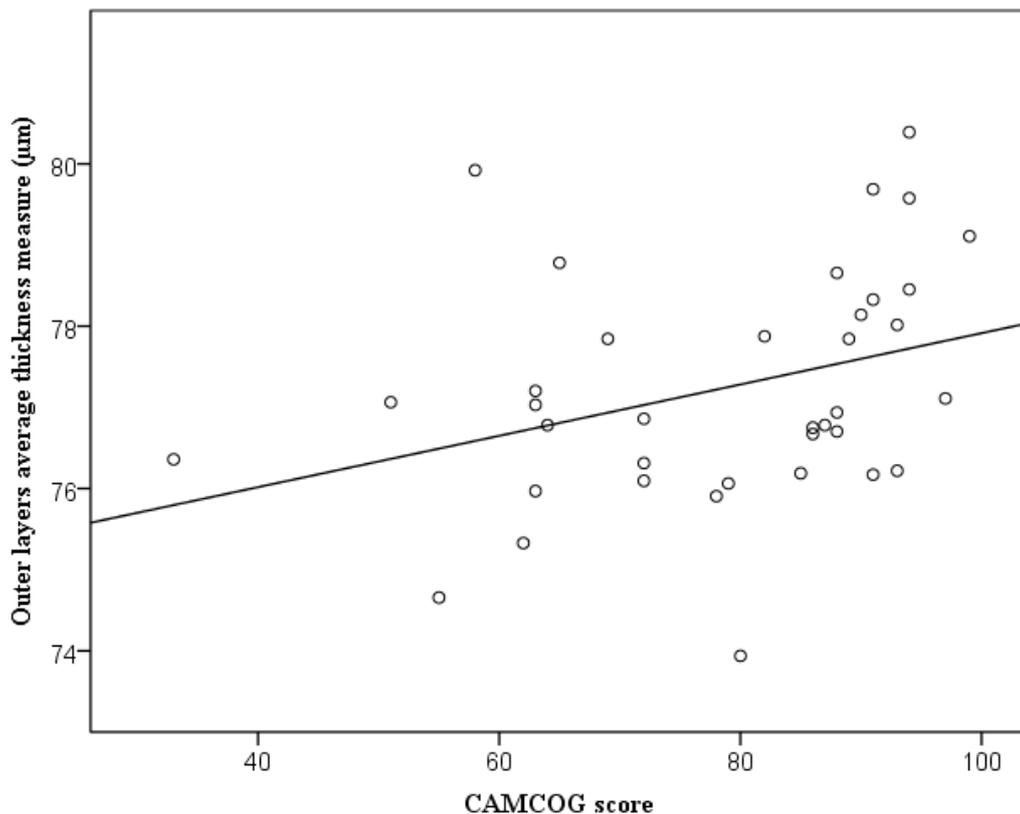


Figure 4.2 Scatterplot showing a positive relationship between CAMCOG score and outer retinal layers thickness.

4.6.4 Cognitive subscale correlations

Previous studies have specifically identified increased inferior quadrant thickness in association with certain cognitive measures other than the MMSE. Individual test component scores within CAMCOG and executive function batteries were correlated against the temporal quadrant thickness. Findings from these correlations showed that there were no significant correlations between the inferior quadrant and the individual measures. Abstract thinking (measured in CAMCOG) showed a non-significant trend towards a positive association ($r = .299$, $p = .046$).

Based on the results of this study, the superior quadrant showed a trend towards an association with the executive function battery, the superior quadrant thickness was also correlated with the individual tests within the batteries. No significant correlations or trends were identified.

4.6.5 Comparisons between groups of DS participants with and without a diagnosis of clinical dementia

Analysis of the CAMDEX informant interviews revealed that five participants met the criteria for dementia. Not all participants in this group had completed all retinal scans; therefore the n for this group is fewer than five for some regions.

	DS with dementia group			DS no dementia group (n = 44)	
	N	Mean ± SD	Range	Mean ± SD	Range
RNFL average	5	117.38 ± 19.07	90 – 134	116.05 ± 12.36	77 – 143
Fovea	3	337.83 ± 20.82	314 – 352	301.36 ± 23.77	231 – 340
Inner macular	3	364.33 ± 9.61	354 – 373	353.98 ± 20.08	304 – 396
Outer macular	3	326.67 ± .14	326 – 327	321.15 ± 15.53	277 – 350
Total layers	4	312.50 ± 12.40	294 – 320	312.97 ± 16.06	263 – 341
Inner layers	4	237.61 ± 12.93	220 – 251	235.67 ± 15.20	189 – 263
Outer layers	4	76.97 ± 2.15	74 – 79	77.17 ± 1.48	74 – 80

Table 4.5 Retinal measure comparisons between DS with dementia group and DS with no dementia group. *Results significant to the Bonferroni adjusted p-value.

All regions of the macular were noticeably thicker in the DS with dementia group when compared to the DS without dementia group, particularly the fovea, which showed a trend towards thicker values in the dementia group ($p = .016$), although only three participants with dementia completed this assessment. The calculated effect size for the fovea region was $r = .39$ which is a medium effect size. Other retinal areas were not significantly different. The number of participants between groups differs substantially therefore the results of this test should be considered with caution.

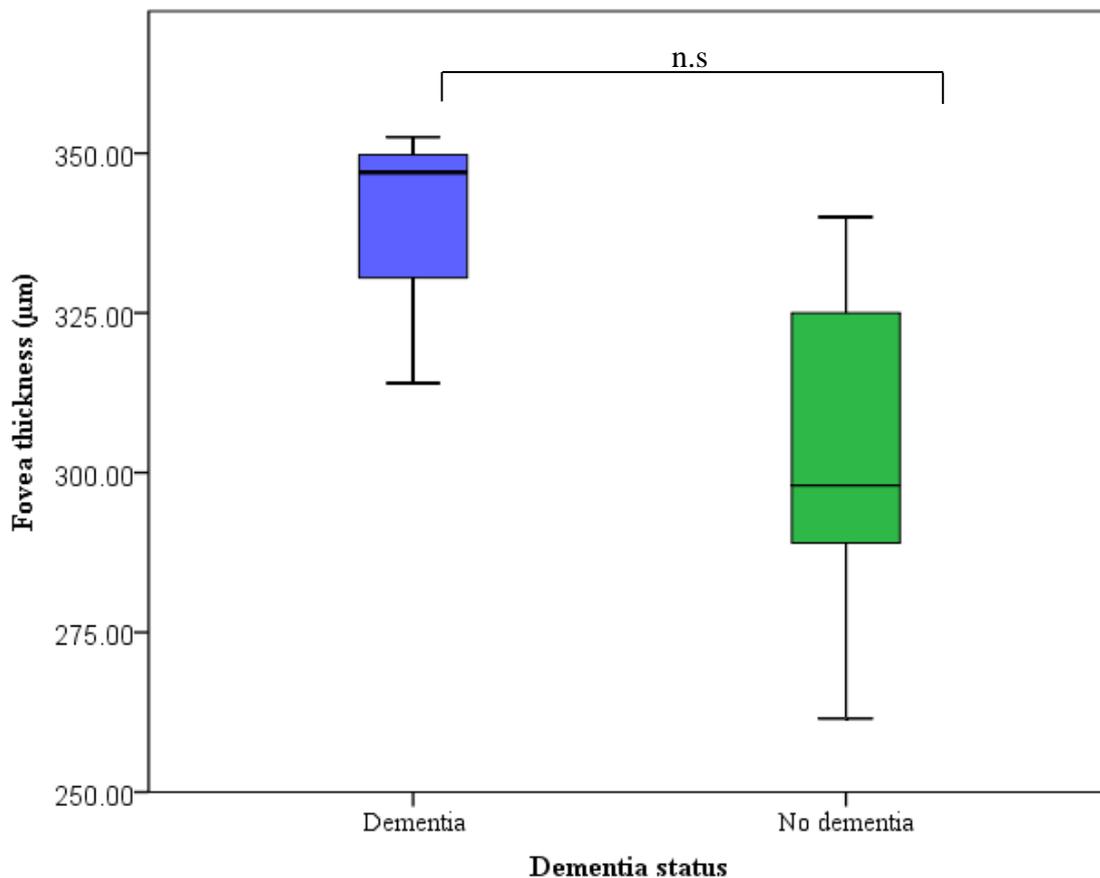


Figure 4.3 Box-plot showing differences in the fovea region thickness between DS with dementia and DS without dementia groups.

The box plot shows a fairly even distribution in the DS without dementia group however in the dementia group there is a clear skew of the mean towards the top end of the results indicating that those diagnosed with dementia have thicker fovea than those without. Again the number of participants in the dementia group is very small therefore these results are tentative.

4.6.6 Comparisons between DS with dementia and an age-and-IQ matched non-dementia DS group

The dementia group was matched with a smaller group of participants from the DS without dementia group. Cases were matched based on IQ and age, please see Table 4.6.

	N	Age Mean (range)	IQ Mean (range)	KBIT-2 raw scores
DS with dementia	5	51.2 (47 – 56)	48.8 (40 – 63)	105 (85 – 133)
DS without dementia	5	46.8 (43 – 55)	50.2 (40 – 65)	102.6 (80 – 128)

Table 4.6 Demographics of the age-and-IQ matched no dementia DS participants to DS participants with dementia.

Of these matched groups, three participants in the dementia group and four in the DS without dementia group completed the fovea scan. Independent sample t-tests showed that there were no significant differences between the groups on age ($t_{(8)} = 1.677$, $p = .132$), or the non-standardised by age KBIT-2 scores ($t_{(8)} = -.206$, $p = .842$).

Fovea thickness was compared between these matched groups (see Table 4.7). It was found that there was not a significant difference between the groups ($t_{(5)} = 2.131$, $p = .086$) but that there was a trend suggesting thicker fovea in the dementia group and again a medium effect size was reported ($r = .38$).

DS groups	N	Fovea mean \pm SD
DS without dementia group	3	311.25 \pm 12.47
Dementia group	4	337.83 \pm 20.82

Table 4.7 Mean and standard deviation of fovea thickness between matched DS groups.

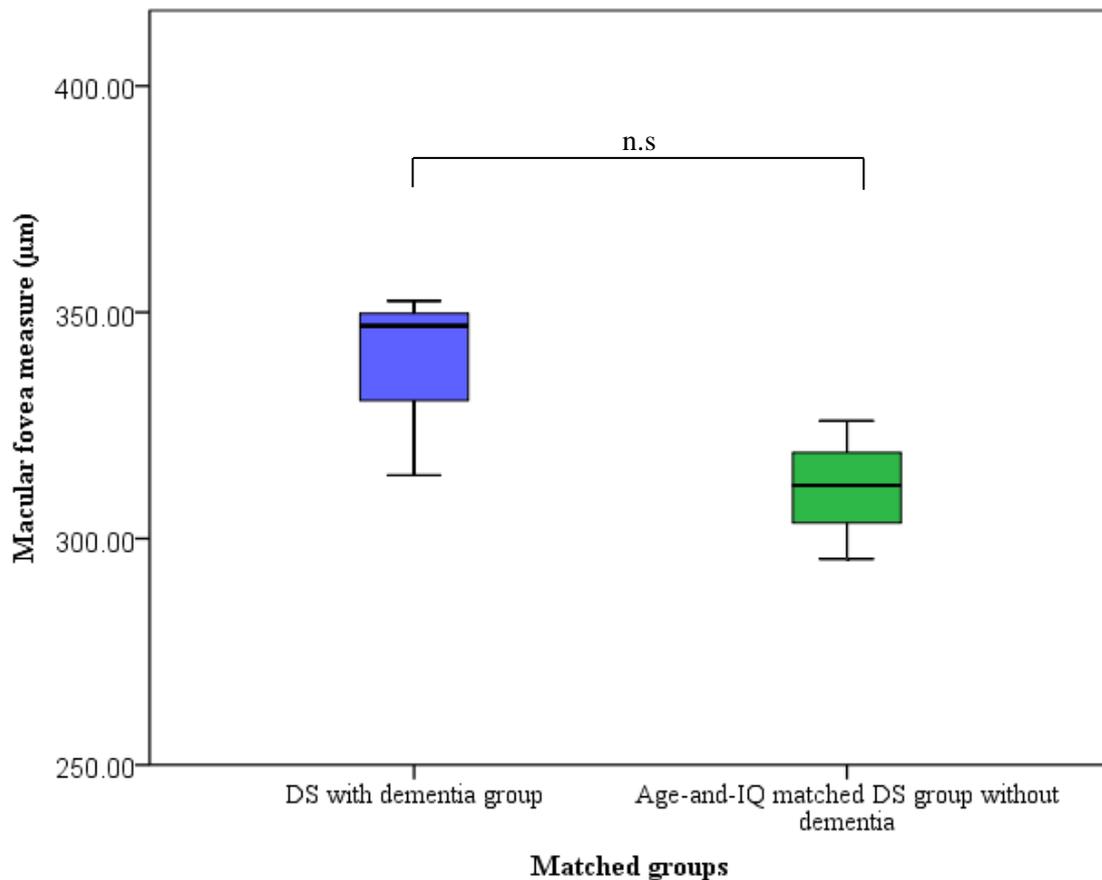


Figure 4.4 Box-plot showing the differences between foveal thicknesses for matched DS groups.

Within the retinal layers and cognitive scores analysis it was identified that the outer layers showed a significant positive relationship with CAMCOG scores. In this analysis outer retinal layer thickness is compared between the dementia and DS without dementia groups using independent samples t-tests.

These comparisons (four participants per matched group; see Table 4.8) showed no significant differences between any of the retinal layers or the retinal average between groups, however, the ONL was found to be thinner in the dementia group ($t_{(6)} = -2.289$, $p = .062$) and a large effect size of $r = .78$ was reported indicating that despite the non-significant p value there is a substantive difference in the ONL thickness of the two groups. The ONL was previously identified both as being thicker in people with DS than controls, and with increasing with age in DS. Non-matched (all DS without dementia) group ($n = 44$), showed no significant differences in the ONL thickness of the dementia group ($n = 4$; $t_{(36)} = -.154$, $p = .879$).

DS groups	N	ONL mean \pm SD
DS without dementia group	4	55.76 \pm 4.36
Dementia group	4	50.66 \pm 0.84

Table 4.8 ONL thickness differences between age-and-IQ matched DS groups with and without dementia.

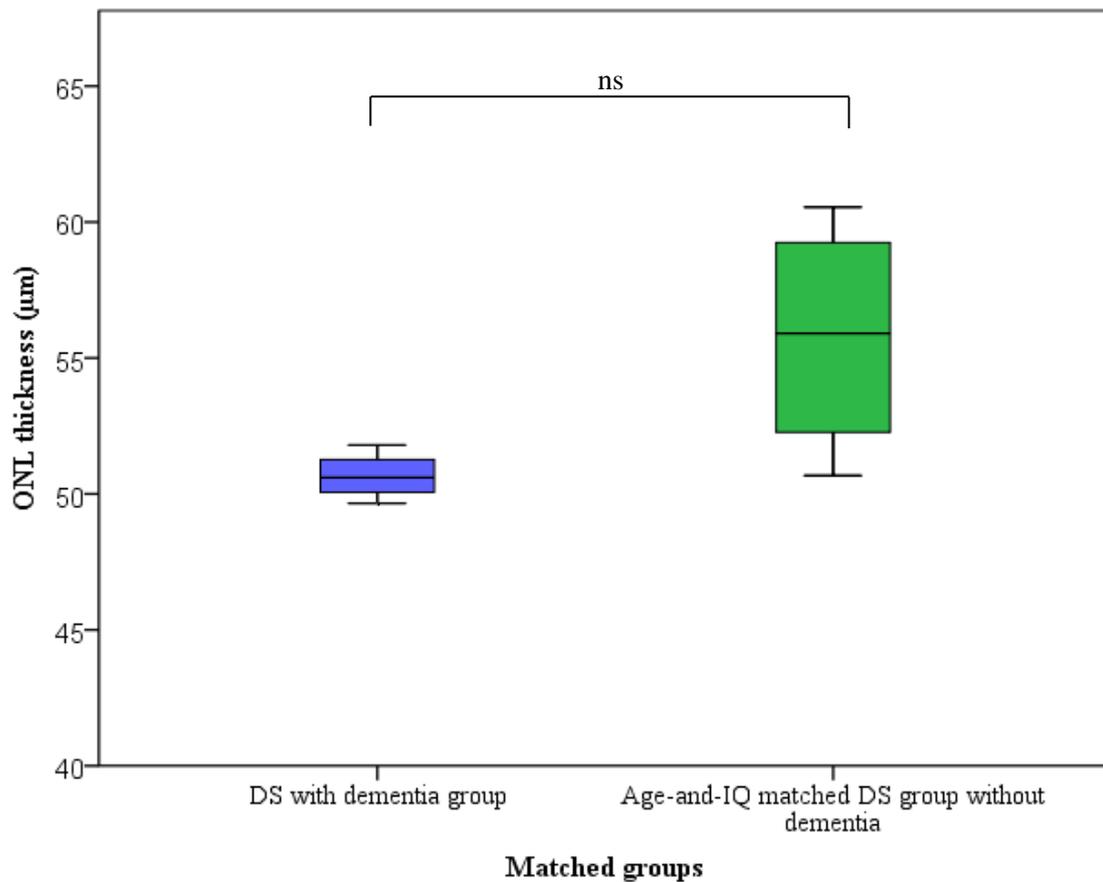


Figure 4.5 ONL thickness spread differences between dementia and no dementia matched DS groups.

4.7 Summary of findings

The aims of this chapter were to examine; (a) correlations between developmental cognitive ability (IQ) and retinal thickness, (b) investigate the association between cognitive task performance and retinal thickness, (c) examine differences in retinal thickness between DS individuals with dementia and those without dementia.

The results of the analyses completed in this chapter showed that retinal measures were not associated with IQ in people with DS, and that, in the majority of cases, retinal measures did not correlate with either of the two cognitive batteries tested. The average thickness of the outer retinal layers showed a significant correlation with CAMCOG scores, indicating that thinner retina was associated with lower cognitive scores. This finding appeared to be strongest for the OPL, although not significant. There were no significant differences found between the DS with dementia and DS without dementia groups in any retinal region apart from the fovea, which was significantly thicker in the dementia group, which is contrary to our predications. It was also found that the outer nuclear retinal layer trended towards thinner values in the dementia group, which would support our hypothesis.

4.8 Discussion

Increased retinal thickness as a developmental feature of people with DS has been suggested, both by the previous results of this study and by studies in children with DS which have found thicker retina when compared to typically developing controls (O'Brien et al., 2015; Weiss et al., 2016). An association between IQ and retinal thickness would indicate that the extent of learning disability is a determining factor in retinal structure. If this were the case, IQ would confound any relationships seen between cognitive performance and retinal thickness. The results of this study did not find any relationship between IQ and retinal thickness. However, this does not remove the possibility that thicker retina is a developmental feature of DS. Future studies should seek to investigate this further.

The finding of thicker fovea in participants with DS who also had a diagnosis of dementia is surprising. It was predicted that those with dementia would show thinner retinal measures, as had been reported in those with sporadic AD (Bambo et al., 2015; Eraslan et al., 2015; Gunes et al., 2015; Iseri et al., 2006; Kang & Kim, 2013; Kirbas et al., 2013; La Morgia et al., 2015; Larrosa et al., 2014; Lu et al., 2010; Marziani et al., 2013; Moreno-Ramos et al.,

2013; Parisi et al., 2001; Polo et al., 2017; Salobrar-García et al., 2016; Salobrar-Garcia, Hoyas, et al., 2015). Studies which have specifically reported foveal thickness found that the fovea was either significantly thinner in AD (Cunha et al., 2016; Garcia-Martin et al., 2014; Iseri et al., 2006), or not different between AD and non-AD groups (Larrosa et al., 2014; Polo et al., 2017). To date, no studies have reported thicker fovea in patients with dementia. Cunha et al., (2016) reported that the strongest correlation with cognition in the retina was in foveal thickness and MMSE scores. Despite the fovea showing a trend towards thicker values in the DS with dementia group, there were no correlations with cognitive test scores.

The outer layers of the retina were the only area that showed any significant relationship with cognition, this was seen with the CAMCOG scores. The layers which make up the outer layer average were assessed individually and only the OPL indicated a trend. Very few studies in sporadic AD have reported on individual retinal layer thickness. One of those which has, Garcia-Martin et al., (2016), found that within the outer layers, only the ONL was significantly thinner in people with AD compared to typically developing controls. In this study, the ONL was thinner in the DS with dementia group, although again numbers in this analysis were very small. The ONL includes the nuclei of the photoreceptors (rods and cones). Photoreceptor loss and damage leads to poor vision including night-blindness and constriction of the visual field (Mitamura et al., 2012). Photoreceptor loss is also linked with ageing. Jackson, Owsley, & Curcio, (2002) reported that by the age of 70 years approximately 30% of central rod photoreceptors would be lost. Other studies have found that specific thinning in the ONL was seen in the ONL after retinal toxicity was induced in a rat model (Fielden et al., 2015). Parnell et al., (2012) reported A β plaque deposition in the ONL and other areas of the retina in an AD mouse model. So far, this study has reported that the ONL is significantly thinner in people with DS compared to age-matched control, found to increase in thickness with age, and be reduced in thickness in those with a diagnosis of dementia.

The inferior quadrant of the RNFL was predicted as a particular area which may show changes associated with cognitive scores, as had been indicated in research in the typical developing population. Knoll et al., (2016) and Shen et al., (2014) both found that thicker RNFL inferior quadrants correlated with poorer cognitive scores and cognitive decline. These studies suggest that some thicker areas of the retina could be associated with onset of dementia, just as Ascaso et al., (2014) found thicker macular in patients with MCI. The

results of this study did not find any correlations between the inferior quadrant and any of the cognitive tests or sub-tests.

4.8.1 Limitations

4.8.1.1 Group size

One of the main limitations from the findings is the very small number of participants in the dementia group. It was attempted to resolve the discrepancy in group sizes by creating a DS group without dementia who were age-and-IQ matched to the dementia group. The groups were successfully matched, however only three and four participants from the dementia group had completed the macular and posterior pole examinations. These small numbers may be the reason why it was not possible to see significant differences between the groups and would need to be replicated in a larger sample. Limitations in our recruitment strategies and other difficulties in recruiting older adults with DS and particularly those with dementia may have influenced this study. These considerations are discussed in more detail in the general limitations section (6.5.3).

4.8.1.2 Comparability of cognitive measures

Cognitive measures used in sporadic AD research and those used in DS are not the same, therefore it is difficult to compare these measures. This study found no differences between CAMCOG scores in those diagnosed with dementia and those without, this could indicate that measures are not sensitive enough to pick up on small changes, individuals were wrongly diagnosed, or that disease progression in the individuals with dementia had not reached a point where cognitive function had been impacted. There are many different approaches taken to diagnosing dementia in people with DS, meaning that across studies there is inconsistency in the methodology applied (Nieuwenhuis-Mark, 2009). The semi-structured informant interview used in this study is recommended by others (Nieuwenhuis-Mark, 2009), however limitations that the interview results are a reflection of one person's opinion of another person's functioning level could have detrimental effects on the validity of the test results.

4.8.1.3 *Preferential sampling within people with DS*

Although the results of this study suggest that retinal thickness is not related to the extent of ID in people with DS, the majority of participants recruited for this study were considered to have a mild-moderate ID. This is likely due to our recruitment pool, particularly those who had taken part in previous studies with the CIDDRG and would therefore have ability levels to be able to comply with the requirements of the studies, and this study also required a level of cooperation that perhaps could not have been achieved in those with severe/profound ID. Had the study captured a wider range of ID it is possible that different results would have been reported.

4.9 Conclusion

Results of this study showed that, generally, there were no associations between retinal thickness measures and IQ or cognitive performance. These findings are generally in agreement with research in typically developing patients with AD, which have mostly found no relationship with MMSE. However in this study, an augmented relationship was expected in the DS group due to the high prevalence of dementia onset and predictable cognitive change after a certain age. Retinal areas, including the inferior and superior RNFL quadrants, which have been explicitly linked to cognitive change in the typically developing population, were not shown to be affected in the DS group.

Non-significant thicker fovea and thinner ONL were found in the DS group with dementia, when compared to the age-and-IQ-matched DS participants without dementia. As discussed in the limitations, a study involving higher numbers of adults with a diagnosis of dementia will be necessary to provide more conclusive evidence. One of the main issues with assessing dementia through changes in behaviour and personality is that this method is only able to capture the later stages of the disease, when clinical assessment has been made. Other technologies, such as neuroimaging are able to provide data on the onset of AD pathology, rather than clinical dementia.

Chapter Five:

Retinal and cortical thickness relationships in the presence and absence of brain amyloid-beta binding

5.1 Chapter introduction

In this chapter, exploratory analyses are undertaken in a subset of retinal imaging study participants with Down's syndrome (DS) who had also taken part in a previous brain imaging study within our research group (Defeat Dementia in Down's syndrome (DiDS)). Neuroimaging data using magnetic resonance imaging (MRI) and positron emission tomography (PET) to investigate measures of cortical thickness and binding potential of A β in the brain were collected as part of this collaborative study. This study was conducted two years prior to the optical coherence tomography (OCT) study. Participants included in the analyses of this chapter completed at least one retinal scan and the brain imaging assessments.

5.2 Background

Alzheimer's disease (AD) manifests in changes which are predominantly seen in the brain, including amyloid-beta (A β) deposits and neural atrophy, which can be visualised using neuroimaging methods such as PET and MRI. A full confirmation of AD can only be made during a post-mortem examination; however neuroimaging techniques are able to provide evidence of AD pathology. Pathology is visible in the brain many years prior to onset of clinical changes, neuroimaging allows for detection at a much earlier disease stage than neuropsychological tests (Lim et al., 2016). The eye, and through it the retina, is the only organ of the body that has the same embryological origins as the brain and can be imaged non-invasively. Studies in patients with sporadic or late-onset AD have investigated whether changes in the eye are linked with AD related brain pathology. Confirmation of a reflection of brain pathology in the retina would enable easier screening and may augment the diagnosis of probable AD, in addition to identifying an easily accessible site for monitoring the outcomes of future treatment trials. In people with DS, there is a unique model of A β -driven AD, which is far less likely to be complicated by aetiological heterogeneity of ageing as is seen in the typically developing population.

MRI provides detailed measurements of regional and whole brain tissue thickness and volume and can be used to quantify brain atrophy, a hallmark of neurodegenerative diseases. Patients with AD have reduced hippocampal and entorhinal cortex volumes, reduced grey matter and cortical thickness and eventual whole brain measurement reduction (Jack et al., 2009, 2010). PET imaging has been developed to be highly specialised in the detection of A β and tau pathology (Bateman et al., 2012). Pittsburgh compound [¹¹C] (PIB) is one of several PET radioligands that bind to fibrillar A β . Using this technology, A β plaques have been identified in typically developing adults over the age of 70 years, up to 10 years before typical clinical onset of dementia. Magnitude and spatial extent of fibrillar A β are associated with older age and the genetic risk for late onset AD (Mintun et al., 2006). Neuroimaging and neuropathology studies have shown that AD pathology presentation in people with DS is very similar to that seen in the typically developing population, (Leverenz & Raskind, 1998; Mann, 1988; Mann & Esiri, 1989), and that A β binding is found in the brain before onset of cognitive or functional decline (Annus et al., 2016; Handen et al., 2012; Hartley et al., 2014). The principle difference in the DS population is that presence of AD neuropathology in the brain is almost certain during the fourth decade (Mann, 1988), regardless of future onset of dementia. In a proof of principle study in people with DS all participants over the age of 45 years had positive PIB binding (Landt et al., 2011), whilst other studies have shown positive binding universally after the age of 39 years (Annus et al., 2016), and 50 years (Hartley et al., 2014).

Analysis completed in this chapter will combine retinal imaging data and neuroimaging data in the same participants with DS. In transgenic AD mouse model research, A β plaques in both the brain and retina have been identified (Koronyo-Hamaoui et al., 2011; Tsai et al., 2014). Koronyo-Hamaoui et al., (2011) quantified the extent of A β in the brains and retinas of a transgenic mouse model of AD and found that plaques were identifiable through curcumin staining at the age of 2.5 months in the retina, and at five months in the brain, hence retinal A β appeared to precede A β in the brain. This finding has not yet been confirmed, Chidlow, Wood, Manavis, Finnie, & Casson, (2017) were unable to support any findings of associations between brain and retinal pathology. One theory is that the sensitivity and increased resolution of OCT has simply allowed for earlier detection, rather than that plaques are forming in the retina earlier in the disease process. In either case, this study showed that there are advantages in imaging the retina in AD, and more sensitive imaging of A β would be valuable. A second part of the Koronyo-Hamaoui et al., (2011)

study included a small pilot study using post-mortem human tissue samples of patients with confirmed AD and probable AD. Findings of this study showed that there was abnormal deposition of A β in the retinas of the AD patients (using curcumin staining) compared to control retinas. Plaques seen in the retina reflected the presence of plaques in the brain. Retinal plaques were also seen in probable AD patients who were considered to be in the early stages of the disease at time of death. Retinal plaques seen in the probable AD patients were described as being more profuse than those which were seen in the brain, which supports the concept that retinal A β may manifest earlier in the retina. Frost et al., (2014) have shown that it is possible to distinguish between PIB positive and PIB negative binding groups based on presence of retinal A β . This study found that there was a strong correlation between the retinal A β index and brain A β burden identified using PET imaging ($r = .762$, $p < .001$).

Changes in brain morphology can also be associated with retinal structure. The cortex shows progressive thinning with age in typically developing adults and further thinning in those with AD (Fortea et al., 2011). Sabuncu et al., (2011) found that thinning cortical regions are a sensitive marker of pre-clinical AD, with an acceleration period occurring during the early stages of the disease. Cortical thickness has been used to differentiate between AD, MCI and non-AD patients (Bakkour, Morris, & Dickerson, 2009; Desikan et al., 2009; Li et al., 2012). Macular volume reduction in patients with AD has been associated with reduced volume in the medial temporal lobe (Casaletto et al., 2017). Results of the collaborative brain imaging study showed that overall thicker cortical regions were seen in the DS participants compared to age-matched controls (Annus et al., 2017). However, participants with DS who were also identified as PIB positive had thinner cortical measures, similar to age-matched typically developing controls, whereas the PIB negative participants had significantly thicker cortices than the control group. Cortical thinning patterns in the PIB positive group were identified as being similar to those seen in non-DS AD patients (Annus et al., 2017).

5.3 Aims

The aim of this chapter is to assess whether the retina is reflective of the brain in relation to changes associated with the onset of AD pathology. Retinal thickness was examined between groups of adults with DS who are identified as having either positive or negative binding of A β in the brain as measured using PIB. Cortical thickness was correlated with retinal thickness and compared between the PIB groups.

5.4 Hypotheses

Hypotheses for this exploratory chapter are based on both literature from the typically developing population in AD and findings from the previous chapters of this thesis and the brain imaging study findings;

- The retina in those participants with DS who have negative PIB binding will be significantly thicker compared to those who are PIB positive.
- The retina in participants with DS who have positive PIB binding will be significantly thicker than the retinas of age-matched typically developing control participants.
- There will be a positive correlation between the thickness of cerebral cortex and that of the retina.

5.5 Methodology

Full details of the retinal scans, data presentation, neuroimaging scans and analysis procedures can be found in Chapter Two, sections 2.7 and 2.9.

The data collected in this chapter was completed in collaboration with another DS project in our research group, the aim of which was to investigate brain atrophy and A β binding in DS adults with relation to AD. This collaborative study used MRI and PET to image the brains of adults with DS over the age of 30 years. This project was conducted as part of the PhD theses of Dr Liam Wilson and Dr Tiina Annus.

Analysis in this chapter is completed in a subset of 18 DS participants who took part in the retinal and brain imaging components of both studies, comparisons to control groups are made for retinal thickness measures only.

5.5.1 Study design

Participants with DS were recruited for the brain imaging study via one of the following sources; clinical and social services for people with intellectual disabilities (ID) in England and Scotland, the Down's syndrome association (DSA), social care providers such as the Home Farm Trust and Craegmoor, and, a number of clinicians in NHS Trusts across England. These services were actively involved in distributing information about the study to individuals with DS and their families.

5.5.1.1 Inclusion criteria

Inclusion and exclusion criteria for the OCT study is as reported in section 2.5.4 Brain imaging study inclusion criteria comprised;

- Aged 30 years or older
- Diagnosis of DS
- Mild to moderate learning disability
- No contraindications for MRI scans
- No co-existing psychiatric illness (e.g. depression), except dementia
- No ferromagnetic implants or other contraindications to having an MRI scan.

5.5.2 Participant demographics

Participants included in the brain imaging study were over 30 years of age and had a clinically confirmed diagnosis of DS. Presence of co-existing active psychiatric illnesses, other than dementia, resulted in exclusion from the study. All participants were screened for MRI safety and individuals with irremovable metallic or ferromagnetic objects were excluded. In total, 18 participants were identified who had useable data from both studies. All participants signed informed consent forms allowing the use of data from both studies collaboratively. Participants were identified as either PIB negative or PIB positive based on

the results of the PET imaging of A β binding, stratification was completed as described in section 2.9.1.2. Demographics of participants whose data are included in these analyses are shown in Table 5.1.

	N	Sex Males, females	Brain scan age (mean, range)	Retinal scan age (mean, range)
<i>Brain imaging time 1 group</i>				
PIB negative	12	8, 4	35.91, 35-46	38.66, 34-37
PIB positive	6	3, 3	49, 39-55	51, 42-56

Table 5.1 Demographics of DS participants who also completed imaging assessments

In both groups there was a significant difference between ages of those in the negative and positive PIB groups ($t_{16} = 4.98, p < .001$). This is expected due to the increased prevalence of A β deposition and AD in older age. There were no significant differences between the genders of the two groups ($T1 \chi = .468, p = .428$).

5.5.3 Neuroimaging scan procedure and analysis

Section 2.9.1 includes a full description of the neuroimaging scans and analysis procedures used. In summary, two complimentary neuroimaging techniques were employed, MRI and PET.

Participants with DS were scanned for 45 minutes using a three Tesla Siemens Verio MRI scanner with 12 channel head coil (Siemens AG, Erlangen, Germany). For all acquisitions the field of view was aligned in stereotactic space with the axial plane aligned to the anterior commissure – posterior commissure line and the sagittal plane to the interhemispheric fissure. For the T₁-weighted structural MRI a magnetisation-prepared, rapid gradient-echo (MPRAGE) sequence was used. This allowed for good contrast between the grey and white matter, and the cerebrospinal fluid (CSF; Mugler & Brookeman, 1990). The following parameters were used: repetition time/ echo time/ inversion time/ flip angle = 2300ms/ 2.29ms/ 900ms/ 9°, 176 slices and a voxel size of 1.0 x 1.0 x 1.0mm³.

For the purposes of the collaboration with this study, cortical thickness measures were analysed. Analysis was performed in FreeSurfer (version 5.3) and calculated using an estimated width of the cortical grey matter (the procedure used can be seen in; Dale, Fischl, & Sereno, 1999 and Fischl & Dale, 2000). Cortical regions were analysed in the temporal, parietal, frontal and occipital lobes for each hemisphere. Paired comparison tests were conducted to identify significant differences between the hemispheres (see appendix S), and data were combined across hemispheres, resulting in a single cortical thickness value for each cortical region. This methodological approach is in keeping with the analysis process of the retinal data.

PET imaging was undertaken on a General Electrical Medical Systems advanced PET scanner with [¹¹C]-PIB and took approximately 90 minutes. Acquired MRI data were used for the co-registration to the PET data. Statistical parametric mapping (version 8) was used to co-register the images and locate the regions of interest. Load and distribution of A β was investigated and participants were assigned to either PIB binding positive, or PIB binding negative groups. PIB positive groups indicated binding potential over two standard deviations of the PIB negative group and more than 0.2 binding level in the striatum. Details of the PIB classification are given in Annus et al., (2016).

5.6 Results

5.6.1 Retinal thickness in the presence and absence of brain A β binding

5.6.1.1 Retinal thickness comparisons between PIB groups

The first aim for this exploratory chapter was to identify the differences between the retinal thicknesses of the PIB negative and PIB positive groups. It was predicted that the PIB positive group would show thinner retina than the PIB negative group. One-way ANOVAs were conducted between the two groups.

Retinal region	N	Mean \pm SD		<i>p</i>
		PIB negative group	PIB positive group	
Global RNFL	12, 5	120.08 \pm 13.25	117.6 \pm 6.79	.700
Fovea	10, 5	312.6 \pm 21.59	325.9 \pm 21.82	.283
Macular inner ring	10, 5	362.59 \pm 14.97	360.80 \pm 18.19	.842
Macular outer ring	10, 5	325.97 \pm 12.56	316.83 \pm 11.25	.193
Inner retinal layers	10, 6	242.13 \pm 12.31	232.64 \pm 12.31	.155
Outer retinal layers	10, 6	78.04 \pm 1.26	77.66 \pm 2.15	.657

Table 5.2 Results of one-way ANOVA assessment of retinal thickness between PIB negative and PIB positive groups.

Results of the retinal thickness comparisons showed that between the PIB positive and PIB negative groups there were no significant differences, however in most regions the PIB positive group had lower average values than the PIB negative group. Only the fovea region in the PIB positive group had a thicker average mean than the PIB negative group. This trend implies that thinner retina in people with DS is associated with presence of A β in the brain; with larger groups a significant result between the retinal thicknesses may be seen. In order to assess this, a sample size calculation was computed using G*Power (version 3.1.9.2; Faul et al., 2009) to determine the sample size required to show significant differences between the two groups. Assuming a large effect size of $D=.08$, $\alpha=.05$ and power = .08 for a one-tailed hypothesis it is recommended that a sample size of 21 participants per group is necessary to attain significant differences between the groups. The present study sample size falls well beneath this target in both groups.

5.6.1.2 Comparison between PIB groups in DS and age-matched controls

The second part of this analysis will consider the differences between PIB groups and control participants age-matched to each of the groups since there was a significant difference between the ages of the PIB groups. To complete this analysis, groups formed of 10 control participants each were age-matched to the PIB negative and PIB positive group separately. There were no significant differences in age for the PIB positive group and their age-

matched control group ($t(14) = -.595, p = .561$) or between the PIB negative group and their age-matched control group ($t(20) = -.486, p = .633$). Global average, macular regions and inner and outer layers of the retina were assessed for differences between groups; results of the independent t-tests are shown in Table 5.3.

	Mean ± SD			Mean ± SD		
	Age-matched			Age-matched		
	PIB negative DS	control group	<i>p</i>	PIB positive DS	control group	<i>p</i>
	group (n=12)	(n=10)		group (n=6)	(n=10)	
Global RNFL	120.08 ± 13.25	94.90 ± 6.09	< .001*	117.60 ± 6.79	97.7 ± 10.54*	.002*
Fovea	312.60 ± 21.59	275.20 ± 18.41	.001*	325.9 ± 21.82	268.70 ± 17.86*	< .001*
Macular inner ring	362.59 ± 14.97	343.90 ± 11.20	.005*	360.80 ± 18.19	339.34 ± 14.11	.025
Macular outer ring	325.97 ± 12.56	304.44 ± 10.75	.001*	316.82 ± 11.25	298.10 ± 15.86	.036
Average layers	316.6 ± 11.98	298.90 ± 11.33	.001*	310.33 ± 13.23	293.6 ± 14.05	.034
Inner layers	242.13 ± 12.31	223.35 ± 11.33	.002*	232.64 ± 12.03	215.31 ± 12.97	.019
Outer layers	78.04 ± 2.34	78.25 ± 2.38	.811	77.66 ± 2.15	78.24 ± 1.69	.554

Table 5.3 Descriptive statistics of PIB negative and PIB positive DS groups' retinal thickness compared to age-matched typically developing control groups. *indicates significant results to Bonferroni adjusted p-value in retinal thickness between PIB group and their age-matched control group.

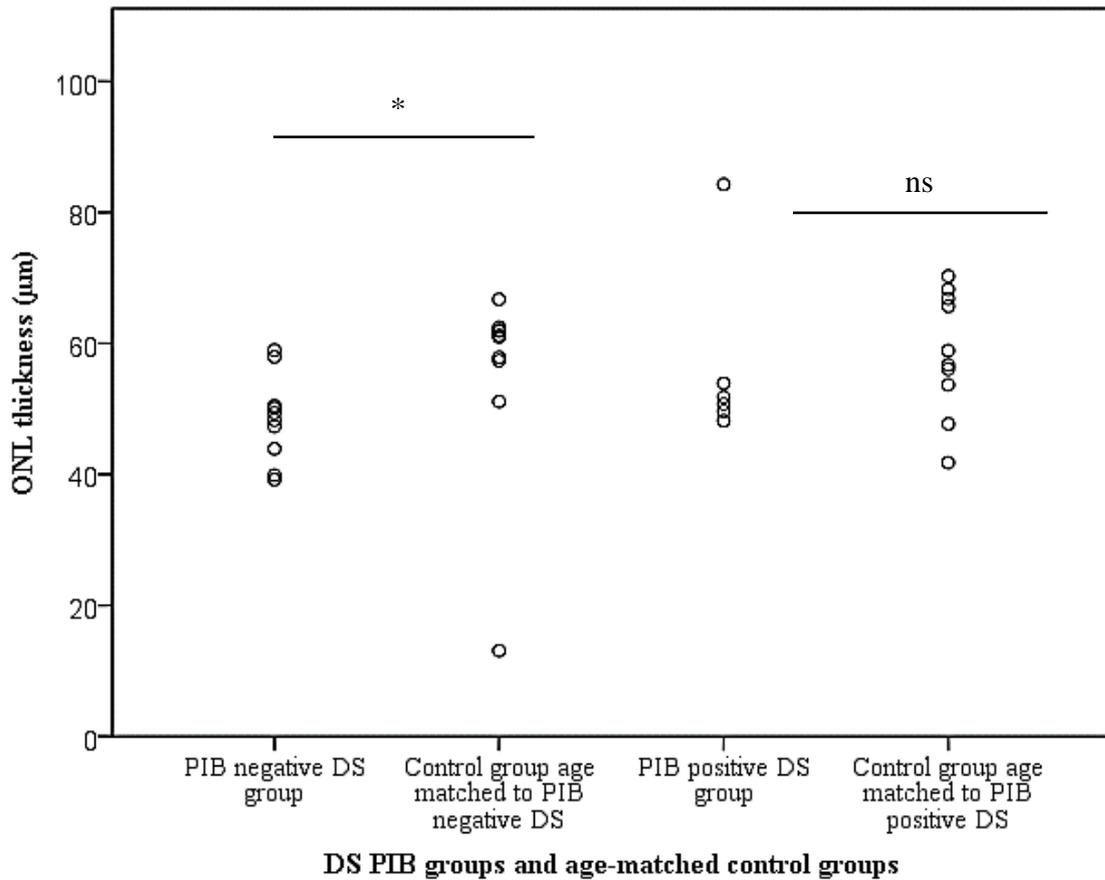


Figure 5.1 Scatterplot showing the outer macular thickness differences between PIB groups and age-matched control groups.

Significantly thicker retina was identified in the PIB negative DS group compared to their age-matched control group. This was significant in all regions apart from the outer layers of the retina. Conversely, the PIB positive DS group did not show significantly thicker retina than their age-matched control group in the majority of retinal areas, significantly thicker retinal measures were only seen in the global RNFL and fovea regions. All other regions were considered to be statistically similar in thickness to the control group. The scatterplot in Figure 5.1 shows the outer macular thickness between the groups.

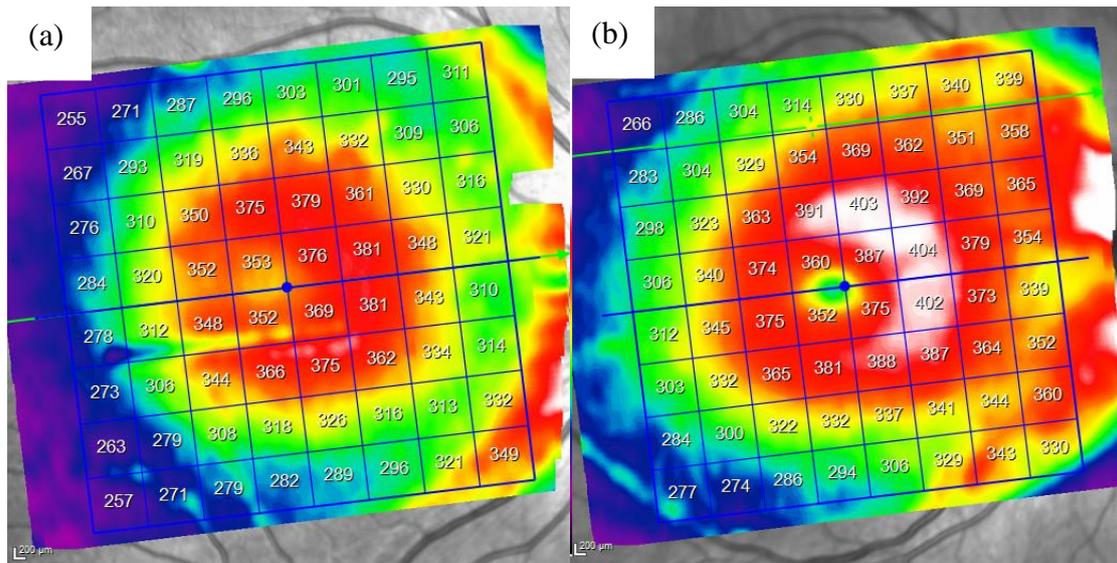


Figure 5.2 Heyex produced colour map showing average retinal layer thickness differences between a PIB positive (a) and PIB negative DS (b) participant of similar age. Colour map represents thicker areas in warmer colours.

Figure 5.2 demonstrates the thickness differences in the average of the total retinal layers between a PIB positive individual (male, 49 years (a)) and a PIB negative individual (male 45 years (b)). The colour map indicates thicker areas in warmer colours. Specifically the macular region of the PIB negative individual appears to be thicker, with warmer colours (red and white) showing in the macular areas. Towards the optic nerve head (ONH) the colour map is much warmer in the PIB negative participant whilst the PIB positive shows a more uniform thickness surrounding the fovea. In the PIB negative participant the fovea region is clearly identifiable in green in the centre, indicating it is thinner than the surrounding macular, however for the PIB positive participant the fovea region is not easily distinguishable from the rest of the macular. This is an example between two participants of how the retina is different between PIB classifications in two individuals and is not generalizable to the whole group.

5.6.2 Relationships between cortical and retinal thickness measures

Bi-variate Pearson's correlations tests between the retinal regions and the cortical thickness regions were conducted. Retinal regions including the RNFL, macular and retinal layers were correlated with the cortical areas, which include the average measures of each of the lobes, frontal, temporal, parietal, occipital and global cortical thickness. The average of both

hemisphere cortices are presented in data analysis in this section, paired comparison t-tests between the two hemispheres were conducted which found very strong associations between the paired lobes, although some significant differences between the hemispheres were identified (see appendix S).

5.6.2.1 Cortical thickness comparisons with RNFL thickness

Results were completed in a top-down method, first correlating each of the cortical regions with only the global RNFL. There was a significant correlation found only between the temporal cortical thickness and the global RNFL thickness therefore quadrant analysis was only conducted for this region. Results are shown in Tables 5.4 and 5.5.

Cortical region	Global RNFL average thickness (n=18)	
	<i>r</i>	<i>p</i>
Global average thickness	.319	.213
Frontal lobe average thickness	.294	.252
Parietal lobe average thickness	-.021	.937
Temporal lobe average thickness	.610	.009*
Occipital lobe average thickness	.097	.710

Table 5.4 Correlations between the global RNFL thickness and the average thickness measure of brain cortical regions.

	Temporal cortical thickness	
	<i>r</i>	<i>p</i>
Global RNFL	.610	.009*
Superior quadrant	.606	.010*
Inferior quadrant	.376	.137
Nasal quadrant	.138	.598
Temporal quadrant	.365	.149

Table 5.5 Correlations between the temporal cortex thickness and RNFL thickness measures. *Results significant to the Bonferroni adjusted p-value.

Quadrant analyses showed that the superior quadrant thickness was significantly correlated with the temporal cortical thickness. Figure 5.3 shows a large positive ($r = .606$) correlation indicating that thicker retina correlated with thicker cortical measures as was predicted.

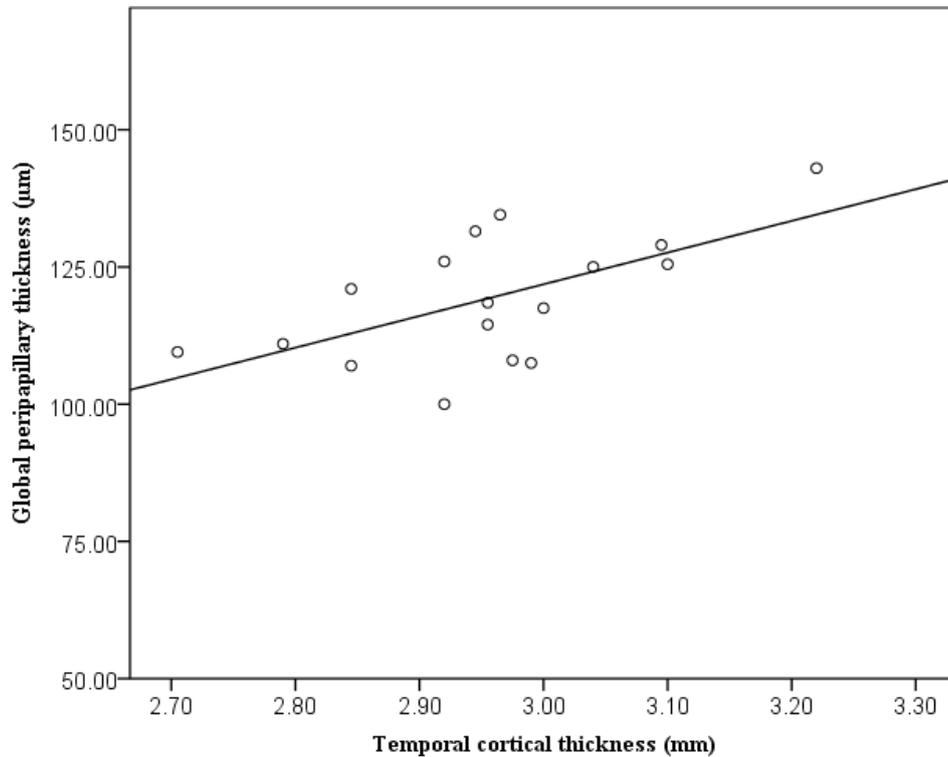


Figure 5.3 Scatterplot showing a significant positive relationship between global RNFL thickness and temporal cortical thickness.

5.6.2.2 Cortical thickness relationship with the macular

There were no significant correlations between the thickness of the fovea, inner or outer macular rings and the thickness of any of the cortical regions. A trend towards significance was seen in the temporal cortical region with the macular outer ring ($r = .591$, $p = .020$).

5.6.2.3 Cortical thickness relationship with the retinal layers

The average thickness of all retinal layers significantly correlated with the thickness of the temporal cortical region ($r = .620$, $p = .010$) and no other cortical regions. The retinal layers were then segmented into the inner and outer layer compositions, the thickness of the inner layers was found to have a significant association with the temporal cortical thickness, ($r = .611$, $p =$

.012) whilst the outer layers showed no correlation ($r = .216$, $p = .421$). The individual layers within the inner layers average were then analysed, results are shown in Table 5.6

	Temporal cortical thickness	
	r	p
RNFL	.556	.025
GCL	.703	.002*
IPL	.622	.010*
INL	.527	.036

Table 5.6 Correlation statistics of the temporal cortical thickness measure with retinal inner layer thicknesses. *Results significant to the Bonferroni adjusted p-value.

Within the inner retinal layers, the thickness of the GCL and IPL showed significant positive correlations with the temporal cortical thickness. Results from this section support our hypothesis that cortical thickness is positively correlated with retinal thickness. Interestingly the only region of the cortex to show significant associations with retinal thickness across all participants with DS was the temporal cortex.

5.6.2.4 *PIB binding dependent relationships between cortical and retinal thickness*

Correlations between the temporal cortex thickness and several retinal regions have been identified in the previous section. This section will consider whether this correlation is impacted by PIB binding status. Global RNFL, macular regions and inner and outer retinal layer averages were computed against all four brain cortical thickness regions within each group.

PIB negative group retinal thickness area						
Cortical thickness area	Global		Inner	Outer	Inner	Outer
	RNFL	Fovea	macular	macular	layers	layers
	(n=12)	(n=10)	(n=10)	(n=10)	(n=10)	(n=10)
Global cortex	.501	-.041	.617	.622	.381	.218
Frontal lobe cortex	.531	-.078	.255	.399	.233	.152
Temporal lobe cortex	.689	.472	.775*	.747	.801*	-.033
Parietal lobe cortex	.108	-.241	.585	.365	.033	.260
Occipital lobe cortex	.095	-.199	.239	.295	.031	.373

Table 5.7 PIB negative group correlations between retinal thickness and cortical thickness. *Results significant to the Bonferroni adjusted p-value.

PIB positive group retinal thickness area						
Cortical thickness area	Global		Inner	Outer	Inner	Outer
	RNFL	Fovea	macular	macular	layers	layers
	(n=5)	(n=5)	(n=5)	(n=5)	(n=6)	(n=6)
Global cortex	-.691	-.917*	-.819*	-.433*	-.226	.464
Frontal lobe cortex	-.695	-.899*	-.878	-.529	-.320	.317
Temporal lobe cortex	-.615*	-.802*	-.577	-.194*	-.057	.728
Parietal lobe cortex	-.730	-.942*	-.833*	-.459	-.166	.296
Occipital lobe cortex	-.256	-.563	-.372	.084	-.018	.647

Table 5.8 PIB positive group correlations between retinal thickness and cortical thickness. *Results significant to the Bonferroni adjusted p-value.

For the PIB negative group the majority of the correlations between retinal and cortical thickness are positive. Again, the temporal cortex is most highly associated with retinal thickness, reporting significant positive correlations in the inner macular, and the inner and outer retinal layers. The outer macular was borderline significant at $p = .013$. Within the retinal layers, the temporal cortical thickness significantly correlated with the GCL thickness ($r = .816$, $p = .004$).

In the PIB positive group the differences in correlations are very striking. In this group, there are very large negative correlations between retinal thickness and cortical thickness, which dominate through all retinal regions apart from the outer retinal layers. Although none of the correlations are significant this is most likely due to the small numbers in this group.

Fisher's r -to- z transformations were conducted to test for differences in the correlations between the groups in the correlation coefficients. Results identified that there were several correlations that were significantly different between the two groups. This was particularly prominent in the fovea region which showed no relationships in the PIB negative group and large negative relationships with cortical thickness for the PIB positive group. Inner and outer macular thickness measures showed differences between the relationships between groups, and the temporal lobe cortical thickness with global RNFL thickness also differed depending on PIB binding status.

Data from this analysis suggests that thicker retina is associated with thinner cortex in people with DS who have positive PIB binding in the brain. In the PIB positive group, neuroimaging and retina analysis have both indicated thinner measures in comparison to the PIB negative group. Longitudinal studies assessing both retinal and cortical thickness would be required to understand how this relationship changed with time and the onset of dementia.

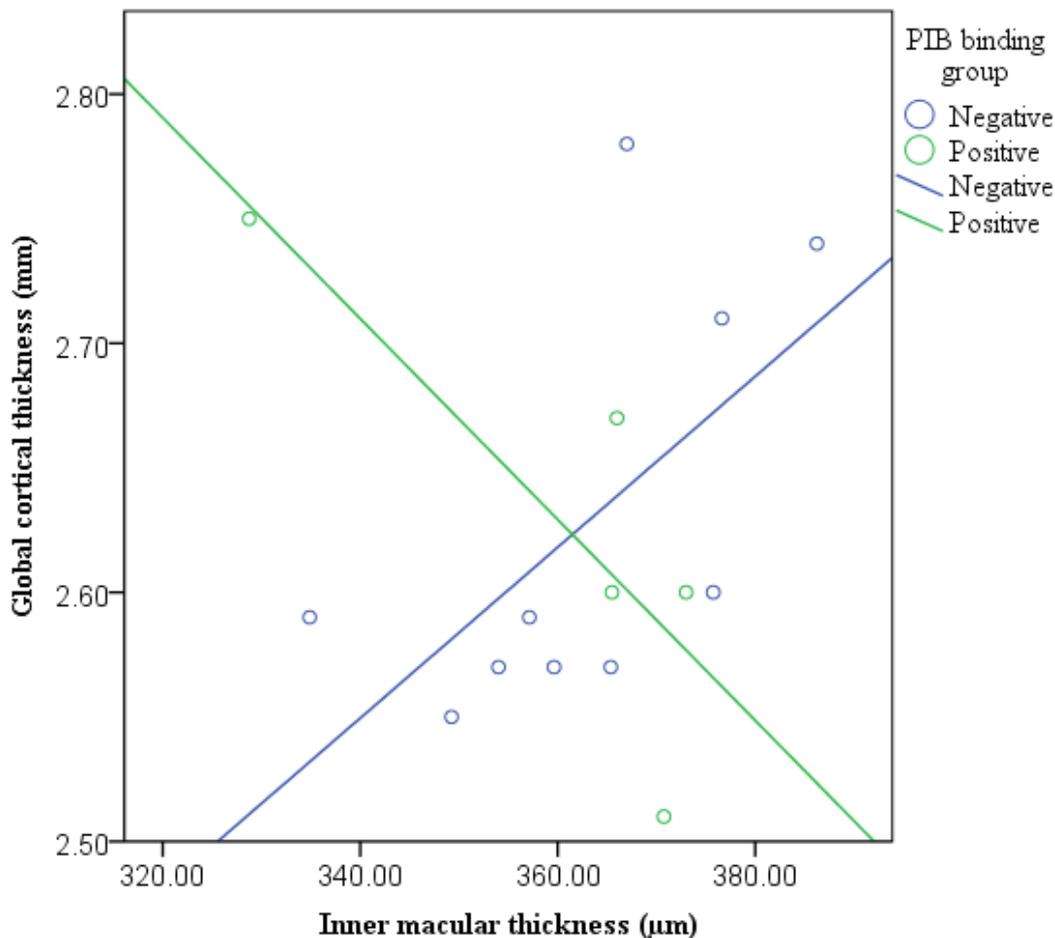


Figure 5.4 Disparity between inner macular thickness correlation with global cortical thickness between PIB negative and PIB positive groups.

5.7 Summary of findings

The aim of this exploratory study was to investigate the relationship between retinal and cortical thickness in adults with DS and to examine whether this was affected by the presence of A β binding in the brain. Results showed that there were positive correlations between retinal and cortical thickness, these were predominantly found in the temporal cortex. It was found that the PIB negative group showed significant positive correlations, whilst the PIB positive group displayed a shift towards negative non-significant correlations. These were significantly different from the correlations of the PIB positive group. Similar to findings of the neuroimaging study, PIB negative DS participants were found to have significantly thicker retina than age-matched controls, whereas PIB positive participants had statistically similar retinal thickness to their age-matched controls. Results imply that individuals who are PIB

positive may have thinner retina, although the PIB negative and PIB positive groups themselves were not significantly different in retinal thickness. In addition to this, cortical and retinal thickness correlations were found to be dramatically different between the PIB positive and PIB negative groups. Despite a trend towards retinal thinning in the PIB positive group, thinner cortex was associated with thicker retina in the PIB positive group, implying that there is increased thinning in the cortex compared to the retina. Interpretations of these results and limitations of the study are discussed below.

5.8 Discussion

The results of this study indicated that there is retinal thinning in people with DS when A β is detectable in the brain using PET neuroimaging. The retinal component of this study was conducted two years after the neuroimaging; therefore A β had been present in the brain for at least two years. As this is a cross-sectional study it is difficult to attribute a causal relationship as to whether there might be parallel and related processes that lead to both retinal and cortical change. However, these results do suggest that AD-related changes in the brain are in some way reflected in the retina in people with DS and future studies should consider this association. Results of this thesis have been unable to identify a link between retinal thickness and ageing in people with DS, which was surprising due to the natural age-related thinning seen in the typically developing population (Bundez et al., 2007; Guedes et al., 2003; Parikh et al., 2007; Poinoosawmy et al., 1997), and accelerated ageing seen in people with DS (Cossarizza et al., 1990). As ageing is intertwined with AD in the DS population it was interesting to find that retinal thinning was identified in the PIB positive group, but not in older DS, which gives support to the idea that retinal changes are linked with the presence of A β , rather than a reflection of increasing age. Despite that there were no significant differences between retinal thickness of the PIB negative and positive groups, it was found that only the PIB negative group showed significantly thicker retina when compared to the age-matched control group. This result is similar to findings in the cortex which showed significantly thicker cortex only in the PIB negative group compared to controls.

The PIB positive group showed statistically similar retinal and cortical thickness to their age-matched control groups. It was not found that people with DS had accelerated age-related retina thinning, or even typical rates of retinal thinning, however, it is unlikely that people with DS are resistant to age-related changes and more likely that the retina is showing the effects of

ageing in a different way. Research in sporadic AD has found that cortical measures in patients with AD are thinner compared to healthy controls (Lerch et al., 2005) and patients with MCI (Bakkour et al., 2009; Desikan et al., 2009; Li et al., 2012). This indicates that cortical thinning could be a subtle measure of AD-related change. Becker et al., (2011) found that clinically normal elderly patients identified as PIB positive showed reduced cortical thickness, particularly in the parietal and cingulate regions. Unpublished data from the DiDS brain imaging study showed similar cortical thickness measures between the PIB positive and age-matched control groups, whereas cortical thickness was significantly thicker in the PIB negative group compared to controls.

Surprisingly, this study showed that specifically the temporal lobe was involved in correlations between retinal and cortical thickness. Given the direct links between the occipital lobe and vision, and that visual areas in the occipital cortex, such as Brodmann area 19 have been found to be reduced in people with glaucoma (Bogorodzki et al., 2014), it may have been more expected to find strong relationships in the occipital cortex, however no significant associations were found between the occipital cortex thickness and retinal thickness. The temporal lobes are involved in memory, and orientation (Dutton, 2003) and damage to this area can result in difficulties recalling visual stimuli, visual agnosia and prosopagnosia. In sporadic AD the temporal lobe is implicated, and also in frontotemporal dementia (Chan et al., 2001; Galton et al., 2001). The temporal lobe shows early signs of volume loss, which has been suggested as marking the beginning of the disease process (Kaye et al., 1997). Excessive atrophy in the temporal cortex may be influenced by the hippocampal region, which is located in the temporal medial lobe and is the first to have atrophy and test positive for A β plaques in late onset AD in the typically developing population.

The brain in people with DS has been shown to be different in both volume and structure to the general population. Reduced volume is typically seen in total brain, frontal lobe and cerebellum (Lee et al., 2016; Mann, 1988; Pearlson et al., 1998), whilst larger percentage volumes have been seen in the parietal and occipital lobes (Beacher et al., 2010; Jernigan, Bellugi, Sowell, Doherty, & Hesselink, 1993). With ageing, greater reduction in brain volume is seen in people with DS than in typically developing controls (Beacher et al., 2010), however some have reported that the temporal lobes are spared from loss (Kesslak, Nagata, Lott, & Nalcioglu, 1994; Strydom, Hassiotis, King, & Livingston, 2009). The temporal lobe, specifically the medial temporal lobe is often a particular focus in AD research as it is a well-known site of atrophy (Burton et al., 2009). The medial temporal lobe includes the hippocampal region,

which is heavily implicated in memory, particularly long term memory (Mormino et al., 2009), which is often more preserved in people with DS. In DS the hippocampus has been shown to be reduced in volume in comparison to the typically developing population (Aylward et al., 1999; Kesslak et al., 1994; Strydom et al., 2009; Teipel et al., 2003). Reduced hippocampal volume has been associated both with increasing age in people with DS, and with dementia in people with DS compared to those without (Kesslak et al., 1994), which may lead to thinning of the temporal lobe.

From the PET imaging results we know that the PIB positive group have A β burden in the brain, however we do not have any data on the A β burden in the retina. Previous studies in this area have found evidence of A β in the retinas of non-demented adults with DS (Rafii et al., 2015) and Koronyo-Hamaoui et al., (2011) have reported A β deposition in the retina prior to its appearance in the brain in typically developing AD. As there is known overproduction of A β in people with DS it is more likely that A β is present in the retina of DS adults than controls, and particularly in those with DS who also show positive A β in the brain. This study has identified that the presence of A β in the brain appears to be associated with changes in the retina, although, due to the time lag between the two studies, it is impossible to say whether retinal changes preceded, coincided with, or occurred after positive A β burden in the brain. Longitudinal studies in people with DS may be able to provide information on the link between retinal thinning and A β in the brain, its association with the progression of dementia. Imaging people with DS is advantageous over retinal imaging in the typically developing population, where thinning of the retina could be caused either by AD pathology or typical ageing, in the DS group retinal thinning does not correlate with ageing. This difference in the mechanisms and course of AD pathology could help to screen for AD in people with DS both earlier and through more cost-effective methods if routine OCT examinations were in place in adults with DS.

5.8.1 Limitations

Time lag between studies

One of the chief limitations of this study is that the neuroimaging and retinal imaging examinations were not completed in parallel. Neuroimaging studies were completed up to two years prior to the collection of the retinal data. Parallel data is vital if we hope to speculate on the trajectory of AD-related changes in the retina and whether any changes here can be seen

before changes in the brain, as has been suggested by some research in sporadic AD patients (Koronyo et al., 2012).

In addition to the time lag, longitudinal studies measuring retinal changes over time are essential to monitor thickness changes. This study has reported that retinal thickness does not decrease with age in the DS population, however, this is based on cross-sectional data of a wide age range and not individuals and therefore these findings cannot truly measure age-related change. Combining retinal scans with longitudinal MRI and PET imaging will be necessary to show the true nature of the relationship between cortical and retinal thickness and how this changes during the onset of AD.

Sample size

Combining the useable data from both studies resulted in a much smaller number of participants than in the previous two chapters. This has the consequence of reducing effect sizes, diminishing significant results and the affecting the generalisability of the data. Particularly problematic was the stratification of participants into the PIB groups, as this resulted in only six participants in the PIB positive group. Many of the results in this group are seen as trends, which is possibly due to the fact that there were so few participants. Further studies with increased participant numbers would be required to validate these findings.

5.9 Conclusion

The results of this chapter have indicated that retinal thickness may be reflective of AD-related pathology in the brain. Results showed that temporal cortex thickness was positively correlated only with the retinal thickness of people with DS who had absence of A β binding in the brain. In those with positive A β binding it was shown that relationships with the temporal cortex were mostly inverted, although these were not significant. In comparing retinal thickness of the PIB negative and PIB positive groups, we were able to identify a trend towards thinner retinal areas in people with positive A β binding, although this appeared to be occurring later than cortical thinning. Although the two groups' retinal thicknesses were not significantly different from one another, only the PIB negative group had significantly thicker retina than age-matched typically developing controls. Previous chapters have shown that the thickness of the retinal areas is not correlated with age; therefore this result is not simply showing age differences in

the DS retina, but appears to be related to the presence of amyloid in the brain. Due to the time lag between the two studies it is not possible to speculate on whether retinal thinning may precede brain A β , future studies and longitudinal assessment would be required to achieve this. However, a shift towards retinal thinning in people with DS may provide a marker of the progression of A β deposition in the brain, which would be an easier, more accessible way to screen those who should be recommended for neuroimaging evaluation.

Chapter Six:

General Discussion

6.1 Overall aims of the project

The purpose of this study was to investigate retinal thickness in adults with Down's syndrome (DS) as a marker of age-related change and Alzheimer's disease (AD) pathology. To achieve this, optical coherence tomography (OCT) examinations of several retinal regions were undertaken in; adults over the age of 18 with DS, an age-matched typically developing group, and, an older comparison group. In order to assess the onset of dementia, cognitive ability and IQ were assessed using a range of neuropsychological tests and an informant interview. Finally, in a subset of participants with DS, relationships between retinal thickness and cortical thickness and presence of amyloid-beta ($A\beta$) binding were explored. Cortical thickness was measured using magnetic resonance imaging (MRI) and presence of $A\beta$ was assessed in the brain using positron emission tomography (PET) as part of a collaborative project within our research group.

The outcome of this study has the potential to recommend the retina as a marker of AD related change in people with DS. Using OCT, this could be more easily, effectively and economically imaged than current brain imaging technologies. This would provide a highly anticipated tool for screening for AD and for monitoring the efficacy of treatment trials.

The aims of the study were:

- To investigate retinal thickness in adults with DS in comparison to age-matched controls and an older age comparison group.
- To assess the relationship between advancing age and retinal thickness in people with DS compared to the relationship seen in typically developing adults.
- To evaluate the correlation between cognitive performance and retinal thickness in people with DS.
- To correlate retinal thickness measures with cortical thickness measures in a subset of people with DS.
- To identify differences in retinal thickness between individuals with DS with (a) a diagnosis of dementia and those without and, (b) a presence of $A\beta$ binding in the brain and those without.

Primary hypotheses:

- People with DS will have thinner retina than age-matched controls and will show more similar thickness to older age typically developing (non-demented) controls.
- Retinal thinning will begin at a younger age in the DS group and will progress at a steeper rate.
- Assessments of cognition will positively correlate with retinal thickness.
- Cortical thickness will positively correlate with retinal thickness.
- Individuals with DS with a diagnosis of dementia, and those with positive binding to A β in the brain will show thinner retinal measures than those without.

6.2 Abstract of main findings

Contrary to our initial hypotheses, this study found that the retina is significantly thicker in people with DS when compared to an age-matched control group, in addition, the retina in people with DS does not show the expected age-related thinning seen in the typically developing population. Investigations with cognitive performance found that retinal thickness was not related to IQ or cognitive performance in people with DS, the latter being measured using tests sensitive to dementia related cognitive change. There were correlations found between retinal and cortical thickness, primarily the temporal cortical region was positively associated with RNFL and macular thickness. Individuals with DS who were identified as having dementia using the CAMDEX informant interview and as showing AD pathology in the brain displayed differences in retinal thickness compared to those who did not show evidence of AD. Those with dementia had thicker fovea and thinner outer nuclear layer (ONL) compared to individuals without evidence of dementia, whilst those with A β binding in the brain showed a shift towards thinner retina compared to those without A β binding.

Key findings

Thicker retina in people with DS in comparison to age-matched typically developing controls and older comparison controls.

Absence of a correlation between advancing age and retinal thinning in the DS group, with one exception of increased retinal thickening in the outer nuclear layer (ONL).

No correlation between cognitive test performance and IQ with retinal thickness.

PIB negative groups showed significantly thicker retinal measures than age-matched controls, whilst PIB positive groups did not show statistically thicker retinal than age-matched controls.

Temporal cortical thickness showed strong positive relationships with the PIB negative group retinal thickness, and no correlations or trends towards a negative relationship with the PIB positive group retinal thickness.

Table 6.1 Summary of the key results of the thesis

6.3 Exploration of findings

6.3.1 Retinal thickness and ageing in adults with Down's syndrome

Studies in the typically developing population have found that patients with AD have significantly thinner retina when compared to age-matched non-AD populations (Bambo et al., 2015; Garcia-Martin et al., 2016; Gunes et al., 2015; Iseri et al., 2006; Kang & Kim, 2013; Salobar-Garcia, Hoyas, et al., 2015). People with DS have a very high prevalence for the early-onset of dementia due to AD (Coppus et al., 2006; Holland, Hon, Huppert, Stevens, & Watson, 1998; Visser, Aldenkamp, Van Huffelen, & Kuilman, 1997) and have signs of early ageing in many aspects of their lives (Oliver & Holland, 1986; Zigman, 2013). Based on this knowledge, it was predicted that adults with DS would have thinner retina when compared to age-matched typically developing controls, and that age-related thinning would start earlier in life and be more pronounced. Contrary to this hypothesis, the results of this study found that adults with DS had significantly thicker retina than both age-matched control participants and the older age comparison group. This finding was homogeneous across almost all areas of the retina. In addition to presenting with thicker retina, the DS group was also found not to show evidence of age-related thinning in the retina. In typically developing adults age-related thinning has been shown throughout life and to accelerate after the age of 50 years (Parikh et al., 2007). The control group data supported this research, showing a tendency towards thinning retina and

some areas of significant thinning when the groups were combined resulting in an age-range of 21-93. The absence of any age-related thinning in the DS group was particularly surprising as it is very unlikely that they would be resistant to ageing in the retina, people with DS typically experience accelerated ageing in most aspects of their physiology. It is therefore suggested that people with DS are experiencing ageing in the retina, but that it manifests in a different way to that which has been seen in the typically developing population.

The majority of retinal thickness research conducted in AD patients has focussed on the peripapillary RNFL, and the four quadrants that this area is divided into. AD-related thinning has most consistently been attributed to the superior, and secondly the inferior quadrants in sporadic AD (Kesler et al., 2011; Lu et al., 2010; Polo et al., 2017). The expectations in this study were that these quadrants would be the first to show thinning in the DS group with increasing age and would show the most difference to the control groups. However, the findings were that the superior and inferior quadrants, as well as the temporal quadrant were significantly thicker in the DS group. Only the nasal quadrant did not show increased thickness in the DS group compared to controls. In sporadic, or late-onset, AD, the nasal quadrant has shown less thinning than other quadrants, it has been found that the nasal quadrant only ever showed significant change in AD alongside thinning in at least the superior quadrant and never independently (Kang & Kim, 2013; Kromer et al., 2014; Larrosa et al., 2014). As we showed that the nasal quadrant was statistically similar in the DS group to the control group it was predicted that this may be a reflection of thinning in this area in the DS group.

This prediction was incorrect, this study found that no retinal areas showed thinning associated with age in the DS group. Control participants showed trends towards thinning retina and significant thinning in some retinal areas across the whole age sample, which serves as a verification that the methodology and analysis was not in some way responsible for these unexpected findings in the DS population. The ONL presented a positive correlational trend towards thicker values with increasing age, this correlation was significantly different to the negative correlation seen in the control group. A significant difference between the associations of the two groups implies that there is an opposite ageing effect occurring in the ONL in people with DS. The ONL is one of the outer retinal layers and is composed mainly of the photoreceptor cells; damage to this area is generally associated with apoptosis. Studies into apoptosis in the ONL have shown changes in Müller cell proliferation and gliosis as a result of the toxicity changes from apoptosis (Whiteley & Peiffer, 2002). Increased numbers of Müller cells and inflammation may be responsible for the increased thickening of this area of the retina,

although this would not explain why retinal thickening seemingly only occurs in this region. The ONL has not typically been analysed in research investigating sporadic AD. In one of the DS cross-sectional studies in children, significantly thicker outer retinal layers were seen in the DS children when compared to age-matched controls (O'Brien et al., 2015). Further delineation into individual layers was not undertaken and this was a cross-sectional study in a fairly limited age range (6-16 years) that could not provide data on changes with time or increasing age. However, this result is, in principle, supportive of our findings.

6.3.2 Retinal thickness associations with cognition

IQ and tests of cognition were conducted in this study and scores were correlated with retinal thickness measures in people with DS. IQ was assessed as a measure of cognitive development and neuropsychological tests as a measure of cognitive performance associated with the onset of dementia. Lower cognitive scores are indicative of cognitive decline, which may be attributable to dementia. As this was a cross-sectional study, cognitive scores across the sample are measured against retinal thickness, rather than cognitive change seen in individuals over time. Due to the extent of learning disability in people with DS it is difficult to disentangle lower cognitive scores associated with onset of dementia and those which are developmental in origin. IQ measures were included in this study to correlate cognitive development with retinal thickness. One of the suggestions for the thicker retina seen in people with DS was that this could be a developmental feature of the disorder. If this were the case, it may be possible to see an association between IQ and retinal thickness; furthermore, relationships between IQ and retinal thickness would suggest that IQ should be included as a confounding variable in people with DS during analysis of the retina. Results of these correlations found that no retinal areas were correlated with IQ suggesting that extent of learning disability is not a factor in the thicker retina seen in DS, this does not however rule out the possibility that a thicker retina could be a developmental characteristic of DS.

It was predicted that cognitive performance would be associated with retinal thickness in DS as a measure of declining abilities linked to dementia. This hypothesis was formed on the basis of previous literature in patients with sporadic AD which has shown that poorer cognitive performance on the Mini Mental State Examination (MMSE; Folstein, Folstein, & McHugh, 1975) is associated with a thinner retina (Ascaso et al., 2014; Cunha et al., 2017; Garcia-Martin et al., 2014; Moreno-Ramos et al., 2013; Oktem et al., 2015; Trebbastoni et al., 2016).

However, the link between cognition and retinal thickness is debated, and some studies have shown no relationship between cognitive score and retinal thickness in AD (Eraslan et al., 2015; Ferrari et al., 2017; Gao et al., 2015; Gharbiya et al., 2014; Gunes et al., 2015; Iseri et al., 2006; Kesler et al., 2011; Kirbas et al., 2013; Kromer et al., 2014; La Morgia et al., 2015; Paquet et al., 2007; Polo et al., 2017). Cognitive performance in the DS group was tested using two separate cognitive batteries, the CAMCOG and an executive test battery. No correlations were found between the IQ and cognitive measures and age. The majority of the retinal thickness measure also did not show any correlation with cognitive performance. The outer retinal layers thickness showed a significant association with the CAMCOG score, indicating that poorer cognition was correlated with thinner retina. This is supportive of the findings in sporadic AD and also particularly interesting as the ONL, which is one of the outer retinal layers, was found to show increased thickness with age. Although the expectation was that thinner retina would be associated with both increasing age and declining cognitive function, this was not found in people with DS. Finding no correlations between cognition and retinal thickness is not overly surprising as the results in sporadic AD have been very varied. In addition, research conducted in AD patients has typically focussed on identifying an association between the RNFL and cognition; therefore it is not altogether possible to relate the findings of this study to previous research. The results of this study suggest that other areas of the retina may also be associated with cognitive change in AD. It was predicted that people with DS would show a stronger relationship between cognitive scores and retinal thickness, based on our initial hypothesis that people with DS would have thinner retina. The unexpected result that people with DS have thicker retina which is unaffected by advancing age reduces the likelihood that a positive relationship with cognitive scores would be seen.

6.3.3 Retinal thickness in the presence of brain A β

Comparisons between DS with dementia and DS without dementia

CAMDEX informant interviews were conducted with the parents or carers of participants with DS. The purpose of these interviews was to make a diagnosis of dementia. In this study, the retinal thicknesses of those identified as having dementia would be compared against those without any indication of dementia. Interviews were completed for all participants in this study; one CAMDEX result was inconclusive and removed from this analysis. Of the remaining participants, five were identified as having dementia. Further comparisons between the groups

were challenging as there were large differences between the dementia and no dementia groups. Results showed that there were differences between the two groups, and that the DS with dementia group displayed a thicker fovea than the DS without dementia group. Due to the disparity between the numbers of the two groups, a group of DS participants without dementia were age and IQ matched to the DS with dementia group, results of the matched groups showed that a trend towards thicker fovea in the dementia group was retained, although no longer significant.

Exploratory analysis was conducted on the outer retinal layers, which had shown a significant increase in thickness with lower cognitive scores. The results of this analysis found that the DS with dementia had thinner ONL thickness than the DS without dementia group. The finding that the ONL was trending towards being thinner in the DS with dementia group was curious, particularly as the outer nuclear layer had previously been shown to increase in thickness both with older age, and lower cognitive scores, factors that increase the likelihood of dementia in people with DS and yet the ONL was not seen to be thicker in the DS with dementia group. As these were very small groups, it was not possible to generalise this result, however the trajectory of the ONL appears to be thus; thinner in people with DS showing gradual thickening with age, thicker in those with lower cognitive scores and thinner in those with clinical dementia (see Figure 6.1). An increase in retinal thickness prior to thinning may be an indication of inflammatory processes, which are seen in many neurodegenerative disorders.

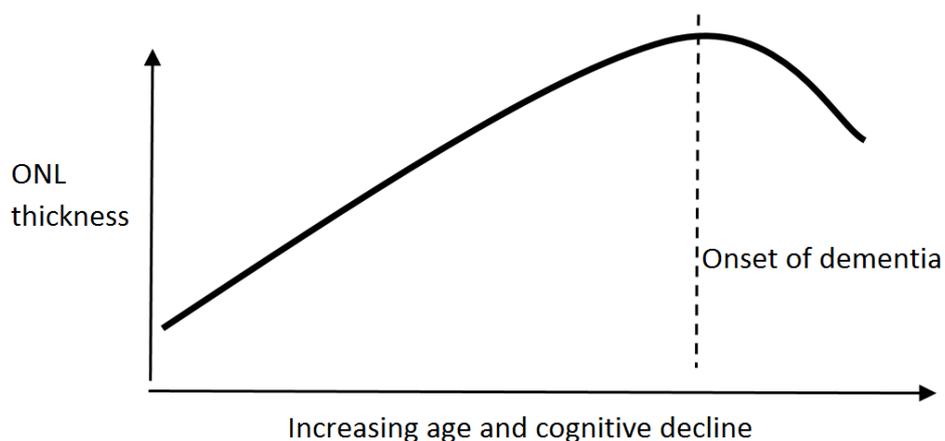


Figure 6.1 Exaggerated predicted trajectory of ONL thickness in relation to age and onset of dementia.

6.3.3.1 *Comparisons between DS with presence of brain A β and those without and further comparisons between retinal thickness and age-matched controls*

Presence of A β in the brain was measured using PET imaging. Stratification of the DS participants involved in this study resulted in 12 participants forming the PIB negative group (indicating binding potential below the threshold of that deemed necessary for positive amyloid binding), and six participants in the PIB positive group. Differences in retinal thickness between the two groups were investigated; the results showed that there were no significant differences in retinal thickness, however, the retina appeared to trend towards being thinner in the PIB positive group. Further assessments against control retinal thicknesses were completed; these analyses found that only PIB negative participants showed significantly thicker cortical regions than age-matched controls. The PIB positive group showed statistically similar retina to the control group in almost every region examined, exempting the fovea and the global RNFL. Although the difference between PIB groups was not significant, there was a clear trend towards thinner retina in the PIB positive group. Significantly thicker fovea in the PIB positive group compared to controls is in agreement with the findings that the fovea was thicker in those diagnosed with clinical dementia.

The results of both this and the neuroimaging study have suggested that there are thinner cortical and retinal measures in people with DS who have positive A β binding in the brain when compared to those without A β binding through comparisons to age-matched control groups. Whilst there was not a statistically significant result between PIB positive and PIB negative groups there is a suggestion that there is a loss of thickness in PIB positive participants. Future studies involving larger groups and specifically more people with DS with positive A β binding and clinical dementia would be needed to corroborate this theory.

6.3.4 Cortical and retinal thickness associations

Cortical thickness was assessed using PET neuroimaging as part of a collaborative study using a subset of the same participants as this study and was correlated with retinal thickness. The results of this previous neuroimaging study found that typically the cortex was thicker in people with DS compared to age-matched controls. The aim of this analysis was to identify whether the retina and the cortex were associated to the extent that thicker values in one correlated with thicker values in another. As previous results in this study had shown that people with DS presented thicker retina it was predicted that there would be a positive relationship between the

cortex and the retina. The thickness measures of the cortex were divided into each of the brain lobes for each hemisphere; the average thickness for both hemispheres was correlated with the average retinal thickness of both eyes. Results showed that there were significant positive correlations between the eye and the brain. Interestingly the temporal cortex was the most implicated in showing significant results with several different areas of the retina.

Correlations between temporal cortical thickness and retinal measures were further investigated between PIB positive and PIB negative groups. The results of this analysis showed that the PIB negative group had strong positive correlations between the cortical and retinal thickness measures in almost every region. However, in the PIB positive group these correlations were different. It was seen that many of the correlations in the PIB positive group had shifted to negative correlations, suggesting that thinner temporal cortex was associated with thicker retina, particularly in the inner and outer macular. Whilst in the PIB negative group, thicker temporal cortex was always associated with thicker retina. None of the correlations in the PIB positive group were significant, although this may partly be due to the small numbers in this group. Analysis of the difference between the correlations of the groups found that there was a significant difference between the PIB negative and PIB positive group correlations suggesting significant change based on A β binding.

6.4 Interpretation of results

The results of this thesis have culminated into two main findings; (a) people with DS have thicker retina than typically developing controls without evidence of age-related thinning, and (b) that a shift towards retinal thinning is seen around the time that an individual presents with A β binding in the brain and presents with cortical thinning. Possible interpretations of these results are discussed below.

6.4.1 Inflammation and A β in the retina

Amyloid plaques

The fact that people with DS have evidence of A β plaques in the brain by the age of 40 years is well established. It has been suggested that people with DS may also have early accumulation of A β in the retina, given the connection between the brain and the retina and the extent of

amyloidosis in people with DS. Rafii et al., (2015) has shown A β in the retinas of people with DS over the age of 30 years using in-vivo imaging technology. It is suggested that retinal amyloid may precede brain amyloid (Koronyo et al., 2012), and could be used as an early marker of AD pathology. In this thesis, retinal A β was not studied, however, it is proposed that the findings seen in retinal thickness may be a consequence of A β pathology both directly and indirectly, and therefore this methodology could provide a proxy measure of A β in the retina.

As previously discussed, one explanation of the thicker retina seen in people with DS is that this is a developmental characteristic of people with DS. Cortical thickness has been shown to be thicker in people with DS compared to age-matched controls (Annus et al., 2017), which is supportive of this theory and the results of this study. However, previous OCT studies in people with DS to date have mainly been conducted in DS children, and these studies are inconclusive as to whether there are retinal differences compared to typically developing controls (O'Brien et al., 2015; Weiss et al., 2016). Even if it were the case that people with DS have a thicker retina as part of their phenotype, this would not explain how this study found that there were no age-related retinal thinning observations in the DS group. This latter finding was contrary to the retinal findings of the age-matched control group, and to the findings seen in age-related cortical thinning shown in many of the same DS participants (Annus et al., 2017). Morphometric studies in humans have shown that changes in early stages of AD are characterised by hypertrophy of the nuclear and cell bodies in the cortex (Fortea et al., 2010; Oh et al., 2009; West, Bach, Söderman, & Jensen, 2009), which has been linked to an early reaction as a result of A β deposition (Iacono et al., 2008, 2009; Riudavets et al., 2009). In the retina, very few studies have found an increase in RNFL and macular thickness with the onset of AD (Ascaso et al., 2014; Salobar-Garcia, Hoyas, et al., 2015). Those that have both attributed increased thickness to very early changes in AD pathology. Ascaso et al., (2014) reports the finding of thicker macular only in patients with mild cognitive impairment (MCI), and that those with AD had significantly thinner retina than both controls and MCI patients. Findings in people with DS saw that the majority of retinal thinning was seen after the presence of A β identified in the brain supports this theory. In people with DS, there is increased likelihood that AD neuropathology has been evident for much longer and starts at a much younger age than in the typically developing population, therefore, seeing changes normally associated with the initial stages of AD for a prolonged period in people with DS may be reflective of the life-long overproduction of A β and subsequent effects. This is also supported

by findings that have suggested that people with DS can tolerate A β deposition without significant effects on their cognitive functioning (Hartley et al., 2014; Rafii et al., 2015).

Research has shown that young children and adolescents with DS have evidence of A β in plasma (Mehta et al., 2007; Teller et al., 1996), and we know overproduction of A β continues through to adulthood as numerous studies have reported the presence of fibrillar A β and A β plaques in the brains of people with DS well before, and in the absence of, a clinical diagnosis of AD. Animals studies using transgenic mouse models of AD have shown that increased deposits of A β in the AD mouse brain (Gupta et al., 2016; Koronyo et al., 2012; Liu et al., 2009; Ning et al., 2008; Perez et al., 2009a), which have not been correlated with retinal neuronal degeneration (Perez et al., 2009). Perez et al., (2009) considered the impact of retinal A β on retinal structure and found that presence of A β was not linked to degeneration of the retinal cells in the AD mouse. However, not all studies have shown evidence of positive A β in the mouse retina (Chidlow et al., 2017; Ho, Troncoso, Knox, Stark, & Eberhart, 2014). In human studies, retinal amyloid has been identified as a result of both post-mortem evaluation (Blanks, Schmidt, et al., 1996; Blanks, Torigoe, et al., 1996; Koronyo-Hamaoui et al., 2011), and more recently in in-vivo imaging (Koronyo et al., 2017). This in-vivo technology has the ability to image retinal amyloid deposits with curcumin and a scanning laser ophthalmoscope, a quantitative measure of increased curcumin fluorescence was constructed to form a calculation of retinal amyloid index. Koronyo et al. (2017) showed in a proof of principle study that the retinal amyloid imaging was increased 2.1-fold in patients with AD versus age-matched controls ($p = .0031$). To date, one study has investigated retinal amyloid in vivo in people with DS, this study identified numerous deposits of A β in the retinas of 12 non-demented adults with DS in comparison to age-matched controls (Rafii et al., 2015). This study identified the presence of retinal amyloid in all 12 DS adults, aged between 32 and 60 years. Retinal A β was detected even in the absence of positive A β in the brain. They were unable to correlate retinal A β with MRI, PET or cognitive data. Retinal thickness analysis was not completed; however, the results of this study might hypothesise that topography of the retina would be thicker in areas with A β deposits. The majority of research has found that AD pathology including A β is present in the retina of both animal and human models, which is supportive that the brain is not the only organ of the body AD in which AD pathology manifests.

Responses to amyloid

A life-long overproduction of A β in the retina may directly lead to the increase in retinal thickness seen in this study; however, amyloid presence may also indirectly cause thickening of the retina. A β is toxic and its presence provokes a neuroprotective response, as A β is deposited in the brain, signals are sent which activate an immunoprotective response including inflammation and increased microglial activity (Dutescu et al., 2009; Gupta et al., 2016; Koronyo-Hamaoui et al., 2011; Tsai et al., 2014). Throughout development microglia play a fundamental role in remodelling the brain by removing redundant apoptotic neurons (Bessis, Bechade, Bernard, & Roumier, 2007; Calderó, Brunet, Ciutat, Hereu, & Esquerda, 2009). Microglia are able to recognize and respond to A β peptides, these cells exert a neuroprotective role through phagocytosis and clearing of soluble forms of A β (Mandrekar-Colucci & Landreth, 2010), however, in the case of A β plaques it has been reported that after increased migration towards the plaques, microglia are unable to degrade the amyloid (Majumdar et al., 2008; Paresce, Chung, & Maxfield, 1997; Walker & Lue, 2005), possibly due to an inflammatory environment which hinders the microglial ability to engage in phagocytosis. This can pose further problems as microglia enters a state of frustrated phagocytosis, causing functional and phenotypic change in the microglia. The interaction of A β and microglia leads to a production of chemokines, and neurotoxic cytokines which are destructive to the CNS (Mandrekar-Colucci & Landreth, 2010) and may serve to increase later degeneration.

In patients with AD, the appearance of A β in the brain coincides with activation of surrounding microglia. Microglia responds to A β deposition and accumulates within and around plaques (Luber-Narod & Rogers, 1988; Rogers, Luber-Narod, Styren, & Civin, 1988), potentially attempting phagocytosis. Physically, microglia increase in number and size in direct proportion to the plaque dimensions (Li, Bushnell, Lee, Perlmutter, & Wong, 1996; Liu et al., 2009; Perez et al., 2009) and are significantly more activated in AD patients (Cagnin et al., 2001; Edison et al., 2008). There is further support that microglia increase degeneration, despite the intent to remove A β , failure to do so results in inflammation. AD brains show increased levels of the inflammatory cytokines and chemokines produced during the interaction of A β and microglia. These have toxic effects on neurons and also insulin degrading enzyme levels, a key A β degrading protease (Mandrekar-Colucci & Landreth, 2010).

Research in diseases such as optic neuritis and Leber hereditary optic neuropathy have found that swelling is followed by a reduction in cell count (Maresca, La Morgia, Caporali, Valentino,

& Carelli, 2012; Serbecic et al., 2011). In the retina, it has been suggested that swelling may represent a pre-apoptotic stage indicating imminent atrophy (Schmitz-Valckenberg et al., 2009). Monocyte-chemotactic protein (MCP-1) attracts macrophages and microglia in retinal disorders, and inflammation from the over-expression of (MCP-1) has been shown in mouse models of AD as a direct response to retinal A β (Ambati et al., 2003; Davies, Eubanks, & Powers, 2006; Yoshida, Yoshida, Ishibashi, Elner, & Elner, 2003). Ning, Cui, To, Hsiao Ashe, & Matsubara, (2008) found that the accumulation of A β was not associated with a reduction in retinal thickness, which supports retinal stability with age in the DS population if A β is responsible for masking age-related thinning. In human patients, Ascaso et al., (2014) found evidence of thicker retina exclusively in patients with MCI, considered to be early stage AD. Retinal thickness in MCI patients was thicker than both controls and AD patients, implying that increased thickness is linked with early pathology. In this study, retinal changes in MCI were accredited to Müller cell swelling, another early response to inflammation. Müller cells support the survival of photoreceptors and neurons, and are responsible for the structural stabilisation of the retina and for modulating immune and inflammatory responses (Bringmann, Pannicke, et al., 2009). Müller cells have swelling properties and become activated by virtually all pathogenic stimuli (Bringmann, Iandiev, et al., 2009; Bringmann & Wiedemann, 2011). Reactive Müller cells are neuroprotective, but they may also stop supporting the neurons and begin contributing to neuronal degeneration. Reactive Müller cell gliosis is thought to represent a cellular attempt to protect the retinal tissue from damage and to promote retinal tissue repair (Bringmann, Pannicke, et al., 2009). The involvement of Müller cells in immune and inflammatory responses may have detrimental effects, after retinal injury the Müller cells upregulate inflammatory factors, which recruit phagocytic monocytes and macrophages and microglial cells to the injured area (Hollborn et al., 2008; Nakazawa et al., 2006). Müller cells draw water from fluid filled spaces outside of the neuroretina and therefore contribute to water influx and cell swelling (Pannicke et al., 2004; Reichenbach et al., 2007).

Further support of swelling and inflammation in human patients with early AD is provided by Salobar-Garcia et al., (2015) who found that sections of the RNFL showed increased thickening which was limited to those with mild-AD and projected to be an early phase of inflammation neural tissue prior to the degenerative process. This study assessed the RNFL by determining 12 sectors, rather than the more typically used quadrant system, and Ascaso et al., (2014) found MCI inflammation in the macular and not in the RNFL. Although the majority of studies have only reported an increase of thinning, in both patients with MCI and AD, it is

possible that subtleties in increased thickness may have been overlooked due to an overwhelming focus on the RNFL and quadrants. In this study, retinal thickness was increased across the entire DS age sample, unlike the seemingly short increased thickness seen in the MCI and mild AD patients. This would fit with the lifelong overproduction of A β in DS that promotes that gradual thickening is caused by A β and by inflammatory responses. It could be the case that the DS retina does in fact demonstrate thinning with age, with the natural loss of healthy cells, but that the overall increase in amyloid and A β responses are concealing the loss of healthy cells, resulting in a retina that seemingly does not change with age.

6.4.2 Apoptosis of retinal cells

Cell death is profoundly associated with neurodegenerative diseases and occurs via multiple pathways, one being apoptosis, which is a form of programmed cell death and is linked with neurodegenerative diseases such as AD. Apoptosis is a naturally occurring process vital to development, cell turnover and immune system functioning. The toxicity of A β causes stress to surrounding cells which results in induced apoptosis (Estus et al., 1997; Hitomi et al., 2004; Li et al., 1996; Tillement, Lecanu, & Papadopoulos, 2011). When the balance of apoptosis is upset, this can contribute to neurodegenerative disease, autoimmune disorders and cancers (Elmore, 2007). During apoptosis the cell undergoes morphological and biochemical changes, including cell shrinkage, chromatin condensation, membrane blebbing and degradation (Cordeiro, Migdal, Bloom, Fitzke, & Moss, 2011). After the process is completed the apoptotic bodies are immediately phagocytosed by neighbouring cells and macrophages, which avoids an inflammatory response in the apoptotic tissue (Williams & Smith, 1993; Wyllie, Kerr, & Currie, 1980).

Increased apoptosis is seen in the retina during neurodegenerative disease (Elmore, 2007) and the onset of excessive apoptosis has been attributed to the toxic effects of A β accumulation, in addition to normal ageing factors (Li, Chan, Lai, & Yew, 1997). Excessive apoptosis eventually leads to cell shrinkage and cell loss, and is considered at least partly responsible for the high neuronal cell death in AD, and other retinal diseases such as glaucoma (Barinaga, 1998; Shimohama, 2000). Apoptosis has been confirmed in post-mortem AD brain tissue, in mouse models (Ning et al., 2008) and in human brains. Smale, Nichols, Brady, Finch, & Horton, (1995) found evidence of apoptosis in seven of eight AD patients' brains tested, and only minimal evidence in one of four control brains. More recently, a novel *in vivo* method,

Detection of Apoptosing Retinal Cells (DARC), has been used in a triple transgenic mouse model of AD (Cordeiro et al., 2010). This model, developed by Cordeiro et al. (2010) is the only model with both A β and tau neuropathology (Sensi, Rapposelli, Frazzini, & Mascetra, 2008). In this model there is an early accumulation of intra-neuronal A β , which is most similar to the development of disease in patients with DS (La Ferla, Green, & Oddo, 2007). Data showed that there was a low level of apoptotic cell death occurring in normal healthy ageing mice, whilst in the AD mice there were significant increases in the relative numbers of retinal ganglion cells in early-phase apoptosis.

Apoptosis assists in the removal of the dead cells by providing membrane coated vesicles, which are easily phagocytized. The removal of the apoptotic cells is crucial to preventing the spread of their contents into the environment (Paus, Rosenbach, Haas, & Czarnetzki, 1993). In people with DS the phagocytosis function has been suggested to be less efficient than in the typically developing population. Barkin, Weston, Humbert, & Sunada, (1980) determined in a study of 14 DS children that there was a diminished response in phagocytosis, a finding which was later supported in further studies in children (Licastro et al., 1990 and Wysocki, Wysocki, & Wierusz-Wysocka, 1987). The gene *Dryk1a*, which is located on chromosome 21 and triplicated in people with DS, has been suggested as prohibiting the natural process of apoptosis (Laguna et al., 2013). This study showed that removal of the additional copy of *Dryk1a* reduced the thickness of the retinal layers in a DS mouse model and therefore it is suggested that inhibition of apoptosis in the retina leads to increased retinal thickness. Conversely, Novo, Garcia, & Lavergne, (1993) did not find a difference in the phagocytosis functioning levels of 12 children with DS compared to matched controls. Novo et al. (1993) indicated that the reason for differences between these studies could be attributed to the diverse range of methods used to assay the phagocytic process. In this study we saw increased thickness in the retinal layers of people with DS, which may be attributable to depositions of A β , and through this, increased levels of apoptosis. Apoptosis is linked with increased inflammation and, if apoptotic cells are not effectively cleared due to reduced phagocytosis functionality, there could be an increased build-up of apoptotic cell bodies in the DS retina disguising the cell loss.

In people with DS, it is more likely than in any other population that AD is driven by A β and therefore resulting in pathology triggered by A β toxicity. Retinal A β seen in DS retinas (Rafii et al., 2015) further support this case. Increased rates of apoptosis are initially accompanied by an inflammatory response, and eventually results in cell loss and subsequently thinner retinal layers (Thomson, Yeo, Waddell, Cameron, & Pal, 2015). This sequence of events could explain

how the retina in people with DS showed stability of thickness until a certain point in the AD pathology, from which time a shift towards thinner retina was seen.

6.5 Limitations

6.5.1 Potential confounds

Confounds of this thesis primarily stem from the common comorbidities with AD in people with DS, including age and developmental cognitive ability (IQ). Age is particularly problematic in the study of AD in DS, there is debate as to whether AD is an inevitable feature of ageing in DS (Ritchie & Kildea, 1995). It is impossible to ignore that there is a strong link between DS and AD and a certainty that people with DS will develop AD pathology (Ball et al., 2006; Holland et al., 1998; Mann & Marcyniuk, 1987). Many changes in people with DS leading to clinical onset of dementia can also be viewed as an exacerbated ageing phenomenon. Therefore it is often difficult to control for age in DS research without subsequently controlling for dementia. Whilst this is certainly a challenge, this study did not find a relationship between ageing and retinal thickness in people with DS. However, there may be other age-related factors effects and processes resulting in a maintained retinal thickness with increasing age.

Retinal thickness was not found to correlate with cognitive performance in this study, it was also established that developmental cognitive ability was not related to retinal thickness. It is possible that people with DS may have characteristically thicker retina. The findings of this study are not conclusive that retinal thickness is not in some way linked to the extent of ID in people with DS, the majority of participants recruited for this study were considered to have a mild-moderate ID. Had the study captured a wider range of ID, including those with severe and profound ID, we may have seen different results.

Co-morbidity of retinal diseases is one of the most challenging factors to research completed in those with sporadic or late-onset AD. Ageing is a risk factor not only for AD but also retinal diseases such as glaucoma and age-related macular degeneration (AMD), both of which have similar presentation to the symptoms identified as AD-related. One of the principal benefits of the DS population is that they have almost 100% assurance of the development of AD-related pathology, unlike the typically developing population, therefore there is a higher assurance that changes seen in the eye are related to AD rather than a retinal disease. Presence of glaucoma, AMD and other retinal diseases were excluded in this study.

6.5.2 Sample size and completion of OCT examinations

The sample size target for this study was originally 30 participants with DS and 30 healthy controls. This is well within the sample ranges seen for OCT studies investigating AD in typically developing patients and larger than those which have used OCT in children with DS. The study had a low dropout rate; however, due to some difficulties in the completion of retinal examinations, the sample size was increased to 50 participants. This increase negated the loss of data due to movement and fixation issues. Due to this data loss, not all participants have a full set of retinal scans, this proved to be increasingly problematic as participants were subdivided into smaller groups, such as those with a confirmation of dementia or those with positive A β binding in the brain.

One of the difficulties for future research will be participant selection, although it was not investigated in depth, failure to fixate, excessive eye movements etc. did not appear to directly correlate with either age or IQ, thereby it will be challenging to predict which participants will struggle with imaging prior to assessment.

6.5.3 Recruitment challenges

There are numerous issues surrounding the recruitment of people with DS for research, which often leads to preferential sampling and recruitment of a particular “type” of individual who volunteers for research studies. Individuals with more severe learning difficulties are less likely to declare an interest in taking part in research, these individuals are also unlikely to be able to cooperate with the demands of research studies, which leads to over-recruitment of individuals with DS with mild-moderate ID, and an under-representation of those with severe/profound ID. In addition to this, there is the possibility that this study has seen a “cohort effect” in the older adults that were recruited to the study. Those with DS who survive into their 50s may be protected against age-related changes and dementia onset.

This study found difficulties in the recruitment of older adults with DS; this was due to several factors. Firstly, our recruitment procedure was greatly assisted by the Down’s syndrome association (DSA), which was formed in 1970. From this date, DS births were automatically registered with the DSA. Due to this, there would have been many individuals over the age of 45 who were not approached through this avenue (as of 2015, when this study began recruitment). Secondly, those over the age of 50 years are substantially more likely to have

clinical dementia, which impacts on the individual's health, personality and mobility. Participants were required to make a trip to either Cambridge or London to undergo testing, which may have been difficult in those with poor health or reduced mobility. Furthermore, people with DS are typically supported by their parents or carers, older individuals with DS will have quite elderly parents, who may be unable to support them with travel. Those who are supported by carers also present difficulties, as often care homes are not equipped to provide one-to-one support and off-site assistance.

Authorities often feel the need to restrict access to individuals with ID in order to protect them, as a vulnerable group, from exploitation and/or harm. These restrictions, whilst they can be necessary, can also result in ID groups being under-represented and understudied in research. The counter-argument to the view that vulnerable groups should be shielded from research, particularly when capacity to consent is uncertain, is that people with ID have the right to choose whether they would like to participate, and should therefore be given the option to refuse (Lai, Elliott, & Ouellette-Kuntz, 2006). By not including individuals with ID, we are potentially denying a group of people from the benefits that might arise from the research (Oliver et al., 2002). This is particularly relevant in AD related research, as people with DS have higher rates of AD diagnosis than any other population group and arguably more to gain from research and treatment trials in AD. Researchers in CIDDRG have undertaken studies investigating the effects of taking part in research on people with ID and their opinions of research (D'Abbrera, Holland, Landt, Stocks-Gee, & Zaman, 2013). This study reported that generally people with ID enjoy taking part in research; they find it interesting and like the idea of helping other people in the future, even when there is no direct benefit to them.

6.5.4 Multiple-comparison corrections

As the areas of the retina were highly correlated with each other there was a strong possibility that significant results could have been seen by chance. To account for this the Bonferroni correction was applied to all data throughout all analyses that reduced the p value in order to increase certainty of a statistically significant result. Applying the Bonferroni correction reduced the acceptable significance value from the uncorrected $p < .05$ to $p < .012$, $p < .007$ and $p < .005$ dependent on the retinal region and the number of comparable areas within each one. The Bonferroni correction controls for alpha error and is considered to be the gold standard for

multiple comparison corrections (Reuter & Montag, 2016). Having applied the Bonferroni correction and only reported significant results as those which met the revised criteria it is presumed that multiple correction concerns are resolved.

6.5.5 Generalisability

As with all research, there are limits as to how far the findings of the study can be extended. In the case of this study a population of adults with DS and typically developing adults were approached to take part in a study that had no benefits to the individual for taking part, including no payment. The participants involved in the study are self-selecting and it must be noted that there could be particular traits of people who take part in research without any benefits to themselves. There is a limited age range in this research, adolescents and children were not studied. Based on the findings of potentially developmentally thicker retina in people with DS it would be beneficial to study these groups in the future. Upper age range is fairly well represented in this study as participants were recruited close in age to the average life expectancy in DS (60 years; Bittles & Glasson, 2004), however fewer participants in the 50s age bracket were recruited than any other decade. In the control group an older age comparison group was recruited to compare DS to older age as well as matched age in order to further assess the accelerated ageing effects in the retina.

6.6 Developing the research

6.6.1 Key improvements for future studies

Based on the limitations discussed in both this chapter and the limitation sections of the results chapters there are several key areas where improvements could be made to benefit future studies in this area.

- Changes to the imaging protocol;
 - For the DS cohort it would be recommended that two OCT examinations were conducted, with a break in between. This is because some participants may benefit from a “practice” and the best quality scans from both sets would be analysed.

- In an attempt to improve the percentages of usable scans it would be recommended to standardise the order of the retinal imaging, particularly starting with the same eye in each participant so that more usable data from the same areas could be gathered.
- More controlled environment of the examination, such as reducing the number of people in the room and more guidance on not distracting the participant during the scan.
- Increased focus on the recruitment of older adults with DS and particularly those with clinical dementia in order to increase the numbers in these groups, and the statistical reliability of analysis.
- Longitudinal retinal imaging to assess age-related change more accurately.
- Brain and retinal imaging to run within a more succinct time frame.

6.7 Future directions

6.7.1 Detection of apoptosing retinal cells

As part of a phase II clinical trial, a study investigating rates of retinal apoptosis in vivo in people with DS was conducted in June 2017. The data for this study was gathered as part of this PhD but due to delays in the project start date it was not possible to analyse and incorporate the findings into this thesis. The purpose of the DARC study was to investigate the natural rates of apoptosis in people with DS compared to several other populations; healthy controls, patients with optic neuritis, patients with glaucoma and patients with age-related macular degeneration (AMD). Rates of apoptosis observed in people with DS were quantified and would be assessed with relation to age, AD diagnosis and cognitive decline. Analysis between the OCT and DARC data investigating a link between retinal thickness and rates of apoptosis will also be investigated. Sixteen people with DS were included in this study, 14 of whom had previous OCT scans and therefore longitudinal OCT data was collected for these participants, cross-sectional OCT data was collected from the other two participants.

Apoptosis is a natural process of cell death and renewal, which increases gradually with age. Increased levels of apoptosis have been studied alongside the onset of neurodegenerative disease and can be induced by the presence of A β , excessive apoptosis eventually leads to irreparable cell loss. As a proxy measure for amyloid in the retina this study predicted that (a) there would be more apoptosis in the retinas of the DS group when compared to the control

group, and, (b) rates of apoptosis would increase with age and with a clinical diagnosis of dementia. This study has the potential outcome of being able to provide a marker for AD related change, and a technology which has the ability to be utilised in the monitoring of future treatment trials targeting both A β and cell degeneration which could then be seen with greater ease than current brain scanning technology.

6.7.2 Investigating A β and further retinal features in Down's syndrome and Alzheimer's disease

As part of the Deep and Frequent Phenotyping study in patients with AD, a retinal imaging component has been approved that will also be conducted in people with DS. I have received funding to begin this project in people with DS commencing in March 2018. The imaging protocol of this study includes many components that are of particular interest in the DS population. Firstly, OCT examinations will be conducted which will both increase the cross-sectional sample of this study and provide longitudinal assessments of those who volunteer for this study, at this time some participants could have a total of three longitudinal OCT assessments over a period of five years. This will allow us to examine the peripheral retina using ultra-wide field imaging. Crucially, A β imaging in the retina will be investigated using NeuroVision imaging technology.

Direct comparison with both a typically developing control group and an AD patient group will be conducted as part of this study. As part of another ongoing study in the CIDDRG, Neuropsychological Assessment of Dementia in Individuals with Intellectual Disabilities, participants with DS will undertake longitudinal MRI and PET assessments over a five-year period. The intentions of this study will be to correlate retinal data where possible for those participants who volunteer for both studies.

6.8 Final conclusions

In conclusion, this thesis aimed to assess retinal thickness in adults with DS and relate this to AD through further investigations of the effects of retinal thickness with age, neuropsychological test performance, and onset of AD as measured by clinical assessments and A β -beta binding data. To achieve these aims, the study measured the RNFL, macular and posterior pole retinal thicknesses, including individual layers for the posterior pole using OCT.

The thesis then correlated these results with age, performance on neuropsychological tests and cortical thickness. Comparisons of retinal thickness between groups were assessed including; comparisons between DS and age-matched typically developing adults and older age adults and between DS groups identified as having positive A β binding in the brain and negative A β binding.

The findings of this thesis have revealed surprising results about the thickness of the retina in DS and its resistance to normal age-related thinning. In addition, areas of retinal thinning have been associated with those with positive A β binding in the brain indicating that presence of A β in at least the brain is reflected in a change in retinal thickness. The findings of this study have opened many other possible avenues of study, and have laid the groundwork for at least two studies, one recently completed and another due to start in 2018. The presence of soluble A β in the eye may not be directly indicative of AD, but would provide a useful marker in a treatment trial. Measures of apoptotic cell death in the retina are a more direct measure of an endpoint in the AD process. The results of these proposed studies will provide further insight into the effects of AD pathology on the retina in people with DS and will further evaluate the use of multiple retinal imaging methodologies tools for the investigation of early AD-related changes in people with DS and for monitoring the results efficacy of treatment trials.

This study has attempted to answer the question of whether retinal thickness may be used as a biomarker in establishing early AD-related change in people with DS. Results of the analyses with the PET data have suggested increased retinal thinning after A β binding is established in the brain, this indicates that retinal changes occur after brain pathology has manifested. However, in this study, people with DS were found to show thicker retina throughout life, the cause of which is not yet understood. There is a possibility that there is a point at which the retina begins to increase in thickness in people with DS that could be representative of other AD-related pathology. Further studies are required to determine the nature of the thicker retina in people with DS in order to understand the potential of the retina as a marker in AD.

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Appendices

A Ethics committee approval



Health Research Authority

NRES Committee East of England - Cambridge Central

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03 November 2014

Miss Madeleine J. Walpert

Cambridge Intellectual and Developmental Disabilities Research Group

Department of Psychiatry, University of Cambridge

Douglas House, 18b Trumpington Road, Cambridge

CB2 8AH

Dear Miss Walpert

Study title:	Retinal cell degeneration as biomarkers in the eyes: a proof of concept study in people with Down's syndrome, a high risk population for Alzheimer's disease
REC reference:	14/EE/1118
Protocol number:	1
IRAS project ID:	135131

Thank you for your letter of 07 October 2014, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the

REC Manager, Miss Jessica Parfremment,

NRESCommittee.EastofEngland-CambridgeCentral@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Mental Capacity Act 2005

I confirm that the committee has approved this research project for the purposes of the Mental Capacity Act 2005. The committee is satisfied that the requirements of section 31 of the Act will be met in relation to research carried out as part of this project on, or in relation to, a person who lacks capacity to consent to taking part in the project.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Copies of advertisement materials for research participants [Healthy control recruitment poster]	1	15 August 2014
Covering letter on headed paper [Cover letter]		15 August 2014
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Provisional insurance letter]	1	13 August 2014
GP/consultant information sheets or letters [GP letter for DS]	1	15 August 2014
GP/consultant information sheets or letters [GP letter for pilot]	1	15 August 2014
GP/consultant information sheets or letters [GP letter for healthy controls]	1	15 August 2014
IRAS Checklist XML [Checklist_20102014]		20 October 2014
Letter from funder [Funding confirmation letter]	1	10 March 2014
Letters of invitation to participant [Letter of invitation to pilot study]	1	15 August 2014
Letters of invitation to participant [Invitation letter for participants new participants]	1	15 August 2014
Letters of invitation to participant [Invitation letter for participants involved in previous studies]	1	15 August 2014
Other [Certificate for DS participants]	1	15 August 2014
Other [End of study letter]	1	15 August 2014
Other [Nominated consultee information sheet]	2	14 October 2014
Other [Personal Consultee Declaration form]	3	20 October 2014
Other [Nominated consultee declaration form]	3	20 October 2014
Participant consent form [Consent form for pilot study]	3	20 October 2014
Participant consent form [Consent form for DS participants for main study]	1	30 September 2014
Participant consent form [Consent form for previous DS participants for main study]	2	20 October 2014

Participant information sheet (PIS) [Participant information sheet for those involved in previous studies, non easy-read version]	3	20 October 2014
Participant information sheet (PIS) [Participant info booklet - previous participants]	1	30 July 2014
Participant information sheet (PIS) [PIS for pilot study non-easy read version]	2	20 October 2014
Participant information sheet (PIS) [Participant information sheet for new participants, non easy-read version]	3	20 October 2014
REC Application Form [REC_Form_18082014]		18 August 2014
REC Application Form [REC_Form_14102014]		14 October 2014
Research protocol or project proposal [Protocol]	1	15 August 2014
Response to Request for Further Information [Response to Response Not Complete]		14 October 2014
Response to Request for Further Information		07 October 2014
Summary CV for Chief Investigator (CI) [Miss Madeleine Walpert CV]	1	15 August 2014
Summary CV for student [Miss Madeleine Walpert CV]	1	15 August 2014
Summary CV for supervisor (student research) [Prof Holland CV]	1	15 August 2014
Validated questionnaire [Paper for the validation of the Arizona cognitive test battery. Questionnaire cannot be provided for copyright reasons]		
Validated questionnaire [SIB]		
Validated questionnaire [CAMDEX]		
Validated questionnaire [Addenbrooke's cognitive examination revised]		
Validated questionnaire [CAMCOG]		
Validated questionnaire [Paper for the validation of the Oliver memory test. Questionnaire cannot be provided for copyright reasons]		
Other [Participant Information Sheet easy-read for pilot study]	2	30 September 2014
Other [Participant Information Sheet easy-read for new participants]	2	30 September 2014
Other [Participant Information Sheet easy-read for previous participants]	2	30 September 2014
Other [Participant Information Booklet for new participants]	1	15 August 2014
Other [Participant Information Booklet for pilot study]	1	15 August 2014
Participant Consent Form [Healthy control]	1	15 August 2014
Participant information sheet (PIS) [Personal consultee]	2	14 October 14

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document “*After ethical review – guidance for researchers*” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:

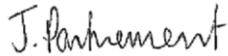
<http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/>

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at <http://www.hra.nhs.uk/hra-training/>

With the Committee's best wishes for the success of this project.

Yours sincerely



Chair Mrs Carolyn Read

Email: NRESCommittee.EastofEngland-CambridgeCentral@nhs.net

Enclosures: “After ethical review – guidance for
researchers” *SL-AR2*

Copy to: *Sponsor/R&D Contact - Mr Stephen Kelleher*

B Participant information sheet for people with Down's syndrome (full version)

Cambridge Intellectual and Developmental Disabilities Research Group

PARTICIPANT INFORMATION SHEET

Eye investigations in Down's syndrome

We would like to ask you if you are interested in taking part in a research study. This study aims to investigate the link between Down's syndrome and Alzheimer's disease by looking at images of the eyes. In this study we will be using optical coherence tomography (OCT) scanning technology, which is used in everyday eye tests at opticians. We are interested in seeing whether this technique can be used to show changes in the eyes which might be able to indicate signs of memory problems. We would like to ask you to read this information explaining what the research study involves and why it is being undertaken.

If you are interested in taking part we will arrange to meet you to discuss the study in more detail and you can ask any questions you have about the study.

This study is being undertaken by Maddie Walpert as part of a doctoral training programme, which is supervised by Prof Tony Holland and Dr Shahid Zaman.

What is the purpose of this study?

People with Down's syndrome have a high risk of developing Alzheimer's disease early in life. Brain scan studies have shown that there may be a link between the changes we find in the brain and changes shown in the retina at the back of the eye. The retina is an extension of the brain and we believe it likely that Alzheimer's disease related changes could be seen here early in the disease development, and by using less invasive methods. It is very important to try and identify signs of Alzheimer's disease as early as possible so that new therapies can be introduced which would have the most effect on slowing the disease. The goal of this study is to develop new ways of identifying markers of Alzheimer's disease at an early stage in development, which will help the development of treatment strategies.

What will happen if I take part in this study?

If you would like to take part in this study, you will be asked to sign a consent form to say that you understand what will happen during the study and that you agree to take part. You can still withdraw from the study at a later stage.

You will be asked to complete some memory puzzles and games and we will also ask your main carer, or the person who knows you best, to answer some questions about you. You and your carer will be asked to come to the hospital for an eye examination using an optical coherence tomography (OCT) scan; which will take around 10 minutes and will require us to dilate your pupils using an eye drop which will increase visibility during the scan. OCT scans are used in everyday eye tests at the opticians. You will be asked to stay as still as possible and look at a flashing blue light while we take pictures of the eyes. We may also need to take a small sample of blood to test for genetic risk factors associated with memory problems in Down's syndrome.

We would like to notify your GP that you are taking part in the study. We will also ask your GP for any confirmation of Down's syndrome tests results they may have and for a copy of your latest thyroid test. If anything unusual is found during any aspect of our investigations we would inform your GP of this.

Will my involvement be confidential?

Any information about you that we collect from this study will be fully anonymised and kept in secure locked filing cabinets. Your name will not be used on any of the data and only members of the research team will be able to see this data. All research information is confidentially stored for 15 years after which it will be disposed of securely.

Blood samples and data collected during this study will be stored securely and may be re-visited in future studies. Your name will not be attached to these samples or data and will remain anonymised.

Will you cover the costs of taking part in this study?

Yes, we will reimburse all travel costs to and from the hospital; we will also cover any food and drink costs while you are visiting the hospital.

What are the risks and benefits of taking part?

There are few risks involved in taking part in this study. The eye examinations will be conducted by a trained ophthalmologist and will feel very similar to when you go to the optician for a normal eye test. These scans may take a little longer as we will be taking high-resolution images of the back of the eyes but they are not at all invasive. The eye drop we will use to dilate your pupils contains tropicamide which is safe although it may cause slight blurred vision, which would wear off shortly after. The blood sample will be taken by a medical professional and as with any blood sample there may be some discomfort involved, this should not last more than a few seconds and we will do everything possible to make this as comfortable as possible. The benefits to taking part are that you will help us learn more about the progression of Alzheimer's disease in people with Down's syndrome and we hope that this will lead to the development of new drugs which can treat Alzheimer's disease at an early stage. If we find anything irregular in your eyes we would be able to inform you and your GP immediately.

Do I have to take part?

No, it is entirely your decision whether you would like to take part in this study. You can withdraw at any time the study at any time and this will not affect your current or future treatment.

What if something goes wrong?

If you wish to complain about any aspect of the way you have been treated during this study you can contact the Patient Advice and Liaison Service (PALS), Addenbrooke's: 01223 216756, Western Eye Hospital: 020 3312 7777. This study has been approved by the Cambridgeshire Research Ethics Committee, and it will then be possible for this research to have insurance cover for negligent and non-negligent harm under the University's Clinical Trials policy.

Contact for further information

If you have any questions before, during or after the research please contact **Maddie Walpert**, who is leading the research, or Professor Tony Holland and Dr Shahid Zaman, who are supervising the research.

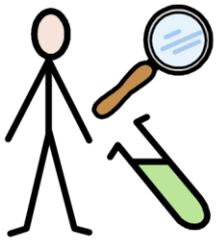
Madeleine Walpert	01223 746172	mjw208@medschl.cam.ac.uk
Dr Shahid Zaman	01223 746100	shz10@medschl.cam.ac.uk
Prof Tony Holland	01223 746112	ajh1008@medschl.cam.ac.uk

Eye investigations in Down's syndrome

Dear



Please read this information carefully and discuss it with others



Researchers at the University of Cambridge and University College London would like to invite you to take part in a study



This study is run by Maddie. She is supervised by Prof Tony Holland and Dr Shahid Zaman

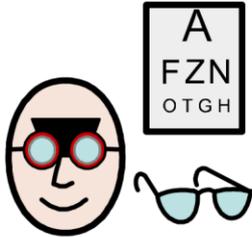


If you are interested in taking part in this study Maddie would come to visit you to discuss it in more detail



What is the research about?

In this study we would like to take photographs of your eyes with a special camera.



We think the eyes might help us to understand why some people get memory problems as they get older



This would be just like having an eye test for new glasses

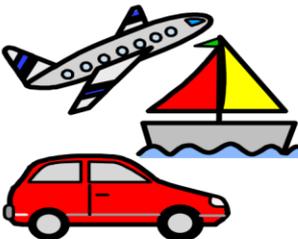


We will use an eye drop to make the scan easier, this may make your vision a little blurry at first

We may need to take a small sample of your blood, this will be taken by a trained professional



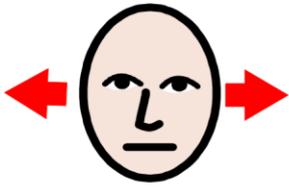
We would ask you to come to the hospital to have this eye test



We will pay for your travel costs to and from the hospital

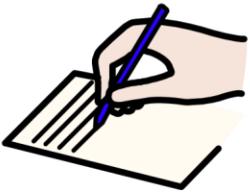


You will need to be at the hospital for a morning or an afternoon. We will do your eye test and some memory puzzles



Deciding whether to take part in this study

It is your choice whether you would like to take part in this study. You do not have to take part



If you would like to take part we will ask you to sign a form saying that you understand what will happen.

You can still change your mind and stop the study at any time



Benefits

By taking part in this study you would help us learn about how the eyes change as people get older



Risks

Taking part in this study will not harm you



All the information about you will be kept confidential and will not have your name on it. You don't need to tell anyone you have taken part in the study if you don't want to. Only anonymised data will be shared with other studies in our group



We have the approval of the Cambridgeshire Research Ethics Committee to do this research



If you are unhappy about the way you have been treated during this study you can talk to Maddie or to the Patient Advice and Liason Service (PALS) for Addenbrooke's hospital on 01223 216756 or the Western Eye hospital on 020 33127777.

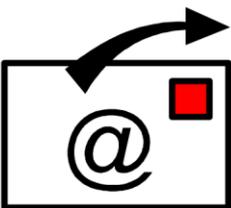


Who can I contact?

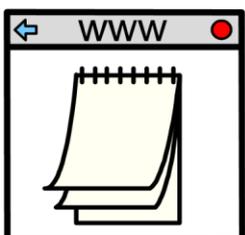
If you have any questions please get in touch with Maddie by phone or email



01223 746172



mjw208@medschl.cam.ac.uk



You can also check out our website for information on all of our studies

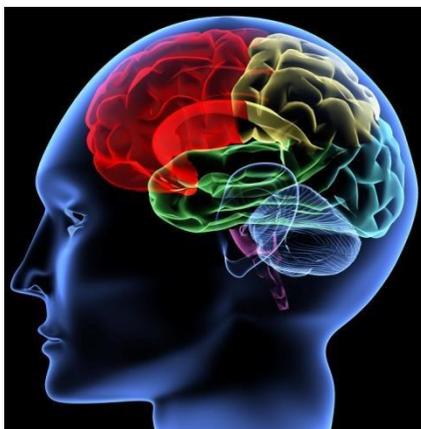
www.defeatdementiains.com

D Picture booklet for people with Down's syndrome

What is this study about?

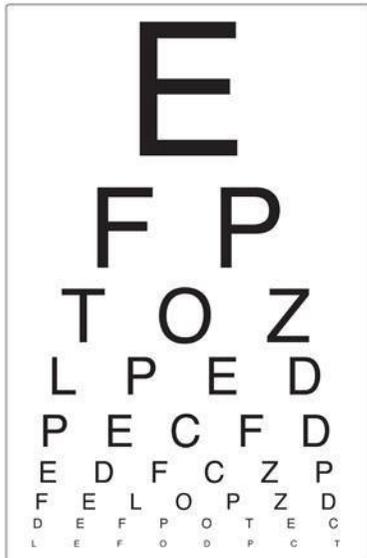


We want to investigate why some people with Down's syndrome get memory problems as they get older.



We think we might be able to see changes in the eyes that will tell us what is happening in the brain.

What will happen?



We will do some tests with you first which will be like those you have when you get new glasses

We will ask you to read some letters

We will look in your eyes to check for cataracts

We will ask you to stare at an object and then follow an object with your eyes

After this we will put a special eye drop in your eyes which will make the scan easier

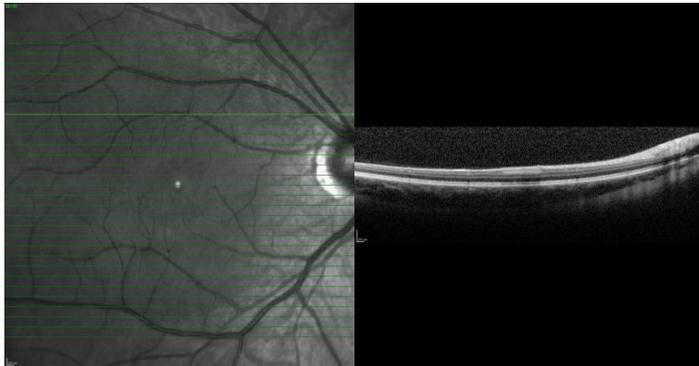
Optical coherence tomography (OCT) scanner



We will use this special scanner to take photographs of the back of your eye

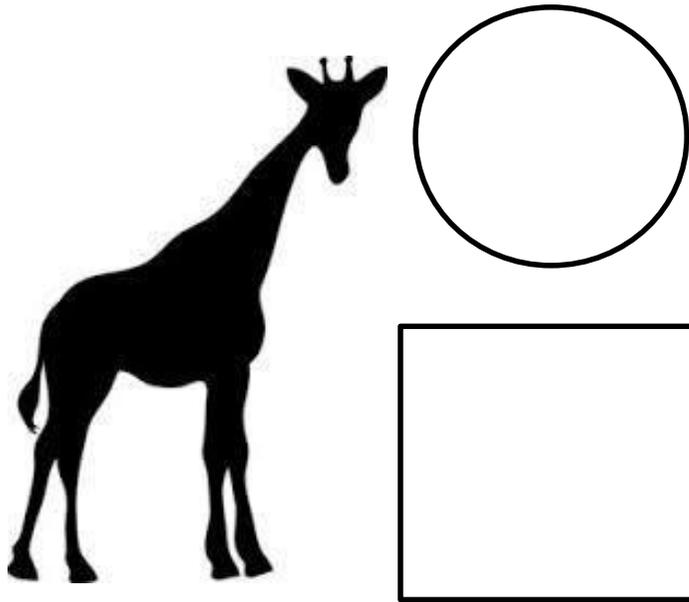
This will take about 5 minutes and you will need to try and stay still and look straight ahead

We will ask you to focus on a flashing blue light



The pictures we take of your eye will look like this one here. These pictures will show us things that are happening at the back of your eyes

Memory puzzles



We will also do some memory puzzles with you and ask you questions about things you like to do.

We would also like to ask your parent or carer questions about the things that you do.



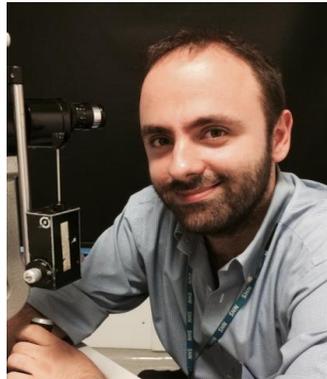
We may also wish to take a blood sample to test for risks associated with dementia, if we do, this would be taken by a trained healthcare professional



Who is involved in the study?

This project is being led by Maddie and is supervised by Prof. Tony Holland and Dr. Shahid Zaman

You will also meet Dr. Eddie Normando who will be taking the pictures of your eyes



Contact information

This booklet is designed to give you some more information about the study. The decision to take part is entirely up to you. If you would like to speak to somebody about the study you can contact Maddie



01223746172



mjw208@cam.ac.uk

You can also check out our website for details of all our studies www.defeatdementiains.com

E Consent form for participants with Down's syndrome

Cambridge Intellectual and Developmental Disabilities Research Group

CONSENT FORM

Study title: Optical coherence tomography assessments in people with Down's syndrome, the link between the eyes and dementia

Please read through the following statements carefully and initial in the corresponding box if you agree



I confirm that I have read and understand the information sheet
date....., version number.....



I have been able to ask questions and discuss the study

I understand that my participation is voluntary and that I can leave the study at any time, without giving any reason and that my care and legal rights will not be affected.



I consent to take part in this study



I understand that parts of my medical notes may be looked at by individuals on the research team. I give permission for these individuals to have access to my records. All the data collected in this study will be anonymised and stored securely for up to 10 years.



I consent to the research team contacting my GP to inform him/her of my participation in this study



I consent to have a blood sample taken

I am happy for my anonymised data to be used in other studies



I consent to the research team contacting me about future studies that I may be interested in taking part in.

Full name:

.....

Date:

.....

Signature:

.....

To be completed by the researcher

Full name:

Date:

Signature:

F Personal consultee information sheet

Cambridge Intellectual and Developmental Disabilities Research Group

PERSONAL CONSULTEE INFORMATION SHEET

We have written to ask N if s/he would be interested in taking part in a research study. This study aims to investigate the link between Down's syndrome and Alzheimer's disease by looking at images of the eyes. We feel that N may be unable to decide for him/herself about whether to take part in the study. As N's main carer, or person who knows them best, we would like to ask you to read this information explaining what the research study involves and why it is being undertaken.

If you decide that N would like to take part in this research you will be asked to sign the personal consultee declaration form, N can still withdraw from the study at any time and this will not affect his or her care in any way. If you have any concerns or think that N should be withdrawn from the study, please let us know. If you are unsure about taking on the role of consultee you may seek independent advice. We will understand if you do not want to take on this responsibility.

Eye investigations in Down's syndrome

This study is investigating age changes in the eyes using an optical coherence tomography (OCT) scan, which are used in everyday eye tests at opticians. We are interested in seeing whether this technique can be used to show changes in the eyes which might be able to indicate signs of memory problems.

This study is being undertaken by Maddie Walpert as part of a doctoral training programme, which is supervised by Prof Tony Holland and Dr Shahid Zaman.

What is the purpose of this study?

People with Down's syndrome have a high risk of developing Alzheimer's disease early in life. Brain scan studies have shown that there may be a link between the changes we find in the brain and changes shown in the retina at the back of the eye. The retina is an extension of the brain and we believe it likely that Alzheimer's disease related changes could be seen here early

in the disease development, and by using less invasive methods. It is very important to try and identify signs of Alzheimer's disease as early as possible so that new therapies can be introduced which would have the most effect on slowing the disease. The goal of this study is to develop new ways of identifying markers of Alzheimer's disease at an early stage in development, which will help the development of treatment strategies.

What will happen if N takes part in this study?

If N does take part in the study s/he will be asked to complete some memory tasks to assess his/her memory function, we will also ask N's main carer, or person who knows them best, to answer some questions about N's behaviour and personality. N will be asked to come to the hospital for an eye examination using an OCT scan; which will take around 10 minutes. OCT scans are used in everyday eye tests at the opticians, and it will be very likely that N has seen this machine before. You would be welcome to stay in the room with N during the eye examination. N will be seated front of the machine which will have a chin and forehead rest, s/he will be asked to stay as still as possible and fixate their gaze on a flashing blue light while we take pictures of his/her eyes. We would also take a small sample of blood to test for genetic risk factors associated with memory problems in Down's syndrome.

We would like to notify N's GP that s/he is taking part in the study and we would ask for consent to do this. We will also ask N's GP for any confirmation of Down's syndrome tests results they may have and for a copy of N's latest thyroid test. If there are any complications during the eye examination we would write to the GP to inform them. Any information about N that we collect from this study will be fully anonymised and kept in secure locked filing cabinets. N's name will not be used on any of the data and only members of the research team will be able to see this data. All research information is confidentially stored for 15 years after which it will be disposed of securely.

Will you cover the costs of taking part in this study?

Yes, we will reimburse all travel costs to and from the hospital; we will also cover any food and drink costs while you are visiting the hospital.

What are the risks and benefits of taking part?

There are few risks involved in taking part in this study. The eye examinations will be conducted by a trained ophthalmologist and will feel very similar to when N goes to the optician for a normal eye test. These scans may take a little longer as we will be taking highresolution images of the back of N's eyes but they are not at all invasive. The blood sample will be taken by a medical professional and as with any blood sample there may be some discomfort involved, this should not last more than a few seconds and we will do everything possible to make this as comfortable as possible.

The benefits to taking part are that N will help us learn more about the progression of Alzheimer's disease in people with Down's syndrome and we hope that this will lead to the development of new drugs which can treat Alzheimer's disease at an early stage. If we find anything irregular in N's eye we would be able to inform you and your GP immediately.

What if something goes wrong?

If N or yourself wishes to complain about any aspect of the way you have been treated during this study you can contact the Patient Advice and Liaison Service (PALS) 01223 216756. This study has been approved by the Cambridgeshire Research Ethics Committee, and it will then be possible for this research to have insurance cover for negligent and non-negligent harm under the University's Clinical Trials policy.

Contact for further information

If you have any questions before, during or after the research please contact **Maddie Walpert** who is leading the research, or Professor Tony Holland and Dr Shahid Zaman, who are supervising the research.

Madeleine Walpert	01223 746172	mjw208@medschl.cam.ac.uk
Dr Shahid Zaman	01223 746100	shz10@medschl.cam.ac.uk
Prof Tony Holland	01223 746112	ajh1008@medschl.cam.ac.uk

G Personal consultee declaration form

Cambridge Intellectual and Developmental Disabilities Research Group

PERSONAL CONSULTTEE DECLARATION FORM

Eye investigations in Down's syndrome

1. I (name of consultee.....) have been consulted about (name of potential participant) taking part in this research study. I have had the opportunity to ask questions about the study and I understand what is involved.
2. In my opinion (name of potential participant) would have no objection to taking part in the above study.
3. I understand that I can request that s/he is withdrawn from the study at any time, without giving any reason and without his/her legal rights being affected.
4. I understand the relevant sections of his/her medical notes and data collected during the study may be looked at by responsible individuals from the Cambridge Intellectual and Developmental Disabilities Research Group or by collaborative researchers or regulatory authorities where it is relevant to my taking part in research.
5. I agree to the GP of the person named being informed of their participation in this study.
6. I consent to the research team keeping samples taken during the study for additional testing and future studies.
7. I consent to the research team looking at data from previous studies the named person may have been involved in with this research group
8. I agree to the Down's syndrome research group contacting the person named above about future studies.

Name of Consultee

Date

Signature

Relationship to participant:

Name of Researcher

Date

Signature

H Nominated consultee information sheet

Cambridge Intellectual and Developmental Disabilities Research Group

NOMINATED CONSULTEE INFORMATION SHEET

We have written to ask N if s/he would be interested in taking part in a research study. This study aims to investigate the link between Down's syndrome and Alzheimer's disease by looking at images of the eyes. We feel that N may be unable to decide for him/herself about whether to take part in the study. As a person or professional who knows N well, we would like to ask you to read this information explaining what the research study involves and why it is being undertaken.

If you decide that N would like to take part in this research you will be asked to sign the nominated consultee declaration form, N can still withdraw from the study at any time and this will not affect his or her care in any way. If you have any concerns or think that N should be withdrawn from the study, please let us know. If you are unsure about taking on the role of consultee you may seek independent advice. We will understand if you do not want to take on this responsibility.

Eye investigations in Down's syndrome

This study is investigating age changes in the eyes using an optical coherence tomography (OCT) scan, which are used in everyday eye tests at opticians. We are interested in seeing whether this technique can be used to show changes in the eyes which might be able to indicate signs of memory problems.

This study is being undertaken by Maddie Walpert as part of a doctoral training programme, which is supervised by Prof Tony Holland and Dr Shahid Zaman.

What is the purpose of this study?

People with Down's syndrome have a high risk of developing Alzheimer's disease early in life. Brain scan studies have shown that there may be a link between the changes we find in the brain and changes shown in the retina at the back of the eye. The retina is an extension of the brain and we believe it likely that Alzheimer's disease related changes could be seen here early

in the disease development, and by using less invasive methods. It is very important to try and identify signs of Alzheimer's disease as early as possible so that new therapies can be introduced which would have the most effect on slowing the disease. The goal of this study is to develop new ways of identifying markers of Alzheimer's disease at an early stage in development, which will help the development of treatment strategies.

What will happen if N takes part in this study?

If N does take part in the study s/he will be asked to complete some memory tasks to assess his/her memory function, we will also ask N's main carer, or person who knows them best, to answer some questions about N's behaviour and personality. N will be asked to come to the hospital for an eye examination using an OCT scan; which will take around 10 minutes. OCT scans are used in everyday eye tests at the opticians, and it will be very likely that N has seen this machine before. You would be welcome to stay in the room with N during the eye examination. N will be seated front of the machine which will have a chin and forehead rest, s/he will be asked to stay as still as possible and fixate their gaze on a flashing blue light while we take pictures of his/her eyes. We would also take a small sample of blood to test for genetic risk factors associated with memory problems in Down's syndrome.

We would like to notify N's GP that s/he is taking part in the study and we would ask for consent to do this. We will also ask N's GP for any confirmation of Down's syndrome tests results they may have and for a copy of N's latest thyroid test. If there are any complications during the eye examination we would write to the GP to inform them. Any information about N that we collect from this study will be fully anonymised and kept in secure locked filing cabinets. N's name will not be used on any of the data and only members of the research team will be able to see this data. All research information is confidentially stored for 15 years after which it will be disposed of securely.

Will you cover the costs of taking part in this study?

Yes, we will reimburse all travel costs to and from the hospital; we will also cover any food and drink costs while you are visiting the hospital.

What are the risks and benefits of taking part?

There are few risks involved in taking part in this study. The eye examinations will be conducted by a trained ophthalmologist and will feel very similar to when N goes to the optician for a normal eye test. These scans may take a little longer as we will be taking highresolution images of the back of N's eyes but they are not at all invasive. The blood sample will be taken by a medical professional and as with any blood sample there may be some discomfort involved, this should not last more than a few seconds and we will do everything possible to make this as comfortable as possible.

The benefits to taking part are that N will help us learn more about the progression of Alzheimer's disease in people with Down's syndrome and we hope that this will lead to the development of new drugs which can treat Alzheimer's disease at an early stage. If we find anything irregular in N's eye we would be able to inform you and your GP immediately.

What if something goes wrong?

If N or yourself wishes to complain about any aspect of the way you have been treated during this study you can contact the Patient Advice and Liaison Service (PALS) 01223 216756. This study has been approved by the Cambridgeshire Research Ethics Committee, and it will then be possible for this research to have insurance cover for negligent and non-negligent harm under the University's Clinical Trials policy.

Contact for further information

If you have any questions before, during or after the research please contact **Maddie Walpert** who is leading the research, or Professor Tony Holland and Dr Shahid Zaman, who are supervising the research.

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Prof Tony Holland	01223 746112	ajh1008@medschl.cam.ac.uk

I Nominated consultee declaration form

Cambridge Intellectual and Developmental Disabilities Research Group

NOMINATED CONSULTEE DECLARATION FORM

Eye investigations in Down's syndrome

1. I (name of consultee.....) have been consulted about (name of potential participant.....) taking part in this research study. I have had the opportunity to ask questions about the study and I understand what is involved.
2. In my opinion (name of potential participant.....) would have no objection to taking part in the above study.
3. I understand that I can request that s/he is withdrawn from the study at any time, without giving any reason and without his/her legal rights being affected.
4. I understand the relevant sections of his/her medical notes and data collected during the study may be looked at by responsible individuals from the Cambridge Intellectual and Developmental Disabilities Research Group or by collaborative researchers or regulatory authorities where it is relevant to my taking part in research.
5. I agree to the GP of the person named being informed of their participation in this study.
6. I consent to the research team keeping samples taken during the study for additional testing and future studies.
7. I consent to the research team looking at data from previous studies the named person may have been involved in with this research group
8. I agree to the Down's syndrome research group contacting the person named above about future studies.

Name of Consultee

Date

Signature

Relationship to participant:

Name of Researcher

Date

Signature

J Participant information sheet for control and comparison participants

Cambridge Intellectual and Developmental Disabilities Research Group

CONTROL PARTICIPANT INFORMATION SHEET

Eye investigations in Down's syndrome

Thank you for your interest in this study. This sheet is designed to give you more information about the study and what will be involved if you do decide to take part. Please feel free to ask any questions you have about the study.

This study is being undertaken by Maddie Walpert as part of a doctoral training programme, which is supervised by Prof Tony Holland and Dr Shahid Zaman.

What is the purpose of this study?

People with Down's syndrome have a high risk of developing Alzheimer's disease early in life. Brain scan studies have shown that there may be a link between the changes we find in the brain and changes shown in the retina at the back of the eye. The retina is an extension of the brain and we believe it likely that Alzheimer's disease related changes could be seen here early in the disease development, and by using less invasive methods. It is very important to try and identify signs of Alzheimer's disease as early as possible so that new therapies can be introduced which would have the most effect on slowing the disease. The goal of this study is to develop new ways of identifying markers of Alzheimer's disease at an early stage in development, which will help the development of treatment strategies.

We would like to ask you, as a person without Down's syndrome, to help make up our control sample. It is important to compare any suspected AD changes seen in a high risk population such as Down's syndrome to a non-high risk population to ensure that any changes seen in the eyes are not part of the typical aging process.

What will happen if I decide to take part in this study?

If you would like to take part in this study you will be invited to the hospital for a visit which may last up to 1 hour. You will be asked to sign a consent form to say that you have read this information sheet and been given the opportunity to ask questions about what will happen during the study.

At the hospital we will ask you to have an optical coherence tomography (OCT) scan of both your eyes. This scan is used in everyday eye tests and is not invasive but we may need to dilate your pupils with an eye drop to allow for better visibility during the examination. It will take around 5-10 minutes to complete these scans and will require you to fixate on a flashing light for around 30 seconds at a time whilst the images are taken. We will also ask to take blood sample from you which will be taken by a trained nurse at the facility. Finally we will do a quick memory test with you to rule out any possibility of dementia.

Within the consent form we will ask your permission to notify your GP that you are involved in the study. We would most likely only notify your GP if the ophthalmologist found something unusual in your eyes during the examination.

Will my involvement be confidential?

Any information we collect from you for this study will be fully anonymised and stored securely in a locked filing cabinet. Your name will not be used on any of the data and only members of the research team will be able to see this data. All research information is stored confidentially for 15 years after which it is disposed of securely.

Blood samples and data collected during this study will be stored and may be re-visited in future studies. Your name will not be attached to these samples or data and will remain anonymised.

Will you cover the costs of me taking part in this study?

Yes, we will reimburse all travel costs to and from the hospital; we will also cover any food and drink costs while you are visiting the hospital.

What are the risks and benefits of taking part?

There are few risks involved in taking part in this study. The eye examinations will be conducted by a trained ophthalmologist and will feel very similar to an eye examination at the optician for a normal eye test. These scans may take a little longer as we will be taking high-resolution images of the back of your eyes but they are not at all invasive. The eye drop we will use to dilate your pupils contains tropicamide which is safe although it may cause slight

blurred vision, which would wear off shortly after. The blood sample will be taken by a medical professional and as with any blood sample there may be some discomfort involved, this should not last more than a few seconds and we will do everything possible to make this as comfortable as possible.

The benefits to taking part are that your involvement will help us learn more about the progression of Alzheimer's disease in people with Down's syndrome and in the general population, we hope that this will lead to the development of new drugs which can treat Alzheimer's disease at an early stage. If we were to find anything irregular in your eyes we would be able to inform you and your GP immediately.

Do I have to take part?

No, it is entirely your decision whether you would like to take part in this study. You can withdraw from the study at any time, and this will not affect your current or future treatment.

What if something goes wrong?

If you wish to complain about any aspect of the way you have been treated during this study you can contact the Patient Advice and Liaison Service (PALS) Addenbrooke's: 01223 216756, Western Eye Hospital: 020 3312 7777. This study has been approved by the Cambridgeshire Research Ethics Committee, and it will then be possible for this research to have insurance cover for negligent and non-negligent harm under the University's Clinical Trials policy.

Contact for further information

If you have any questions before, during or after the research please contact **Maddie Walpert** who is leading the research, or Professor Tony Holland and Dr Shahid Zaman, who are supervising the research.

Madeleine Walpert	01223 746172	mjw208@medschl.cam.ac.uk
Dr Shahid Zaman	01223 746100	shz10@medschl.cam.ac.uk
Prof Tony Holland	01223 746112	ajh1008@medschl.cam.ac.uk

K Consent form for control and comparison participants

Cambridge Intellectual and Developmental Disabilities Research Group

CONSENT FORM

Study title: Optical coherence tomography assessments in people with Down’s syndrome, the link between the eyes and dementia

Please read through the following statements carefully and initial in the corresponding box if you agree

1. I confirm that I have read and understand the information sheet
date....., version number.....
2. I have been given the opportunity to ask questions and discuss the study
3. I understand that my participation is voluntary and that I have the right to withdraw at any time, without reason and that my medical care and legal rights will not be affected.
4. I consent to take part in this study
5. I understand that sections of my medical notes may be looked at by responsible individuals from the Cambridge Intellectual and Developmental Disabilities Research Group or by collaborative researchers or regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. All the data collected in this study will be anonymised and stored securely for up to 10 years.
6. I consent to the research team contacting my GP to inform him/her of my participation in this study
7. I consent to have a blood sample taken
8. I am happy for my anonymised data to be used in other studies
9. I consent to the research team contacting me about future studies that I may be interested in taking part in.

Full name:

.....

Date:

.....

Signature:

.....

To be completed by the researcher

Full name:

.....

Date:

.....

Signature:

.....

L Sample size calculation and justification

At the time of the study proposal we were aware of one meta-analysis of studies comparing RNFL thickness in those with AD compared to controls without AD – He et al., (2012). However, in this paper, only seven studies were finally considered, further, the authors find that there is significant heterogeneity among the results of the studies considered ($\chi^2=62.96$, $p<0.0001$), suggesting important differences between studies. For the purpose of this sample size calculation we have considered the studies listed in He et al., (2012) and several others (see Table L.1), and selected the study that most closely resembled the planned study.

Moschos, Chatziralli and Moschos (2012) was selected, as this study matched control participants to the patient group on age and sex (as is intended in this study), and importantly, had the largest range of ages. The proposed study will recruit participants with DS from 18 years, with no upper limit (expected is 60s), therefore powering from a study with a similarly wide age range (42-84, the widest comparable range) is reasonable.

Basing a sample size calculation on Moschos et al., (2012) is non-trivial as they use a Mann-Whitney U test to compare RNFL between groups, rather than the more ubiquitous two sample t-test. First, we determined the effect size of the difference observed between those with AD and controls. The proposed study will look at average RNFL thickness and the four measurement sites in the RNFL (superior, inferior, temporal and nasal) whilst in Moschos et al., (2012) these four sites were compared separately on left and right eyes. From these eight comparisons, Z-statistics values are produced for each (all indicating a significant thickness reduction in those with AD); we take the median Z-value (-4.21) as an indication of effect size. This can be converted to a correlation measure of effect size r , using the formula given by Rosenthal (1991): $r=Z/\sqrt{N}$, where N is the total sample size being compared in the Mann-Whitney U test. Thus $r=4.21/\sqrt{(60)}=0.54$. In turn, this correlation can be converted to Cohen's D, giving $D=1.28$, using a formula given in Rosenthal (1994), as implemented in Decoster's spreadsheet for converting between effect sizes (Decoster, 2012).

We now calculate a sample size for a (two tailed) two-sample t-test comparison. Given the above comparison we conservatively assume an effect size of $D=1$, along with $\alpha=0.05$ and power=0.9 and calculate (using G*Power version 3.1.9.2, as described in Faul et al., (2007)) a sample size of 23 per group. Given the use of the non-parametric Mann-Whitney U test in Moschos et al., (2012), we inflate this sample size to 24 per group (based on the asymptotic relative efficiency of $3/\pi$ of the Mann-Whitney compared to the t-test, as reported in Lehmann

(1975): $23/(3/\pi) \approx 24$). Finally we assume a drop-out/loss of information of 20% indicating a need to recruit a minimum of 30 people per group ($30/0.8 \approx 38$), giving a total recruitment of 90 people.

Author	Control group		AD patient group		MCI Patient group		Matching	
	N	Mean age in years (s.d)	N	Mean age in years (s.d)	N	Mean age in years (s.d)	Age matched	Sex matched
Ascaso et al., (2014)	41	72.9 (7.9)	18	72.1 (8.7)	21	72.1 (8.7)	Yes	Yes
Berisha et al., (2007)	9	74.3 (5.8)	8	74.3 (3.3)	N/A		Yes	Not clear
Garcia-Martin et al., (2014)	28	72.1 (5.1)	20	79.3 (4.1)			Yes	Yes
Gharbiya, Trebbastoni, Parisi, & Manganiello, (2014)	21	70.3 (7.3)	21	73.1 (6.9)			Yes	Yes
Iseri, Altinaş, Tokay, & Yüksel, (2006)	15	65.1 (9.8)	14	70.1 (9.7)			Yes	Yes
Kesler, Vakhapova, Korczyn, Naftaliev, & Neudorfer, (2011)	24	70.9 (9.2)	30	73.7 (9.9)	34	71.1 (10)	Yes	Not clear
Kirbas, Turkyilmaz, Anlar, Tufekci, & Durmus, (2013)	40	68.9 (5.1)	40	69.3 (4.9)	N/A		Yes	Yes
Larrosa et al., (2014)	61	55 – 91 (age range)	22	66 – 88 (age range)			Yes	Yes

Moreno-Ramos, Benito-León, Villarejo, & Bermejo-Pareja, (2013)	10	70.2 (5.5)	10	73 (6.5)			Yes	Yes
Moschos et al., (2012)	30	Age matched to AD group	30	42 – 84 (age range)			Yes	Yes
Paquet et al., (2007)	15	75.5 (5.1)	12 14*	78.8 (4.9) 78.3 (5.1)	23	78.7 (6.2)	Yes	Not clear
Parisi et al., (2001)	14	Not given	17	70.37 (6.1)	N/A		Yes	Not clear
Polo et al., (2014)	75	73.98 (9.05)	60	74.15 (9.5)			Yes	Not clear

Table L.1 Literature reviewed for the sample size calculation * For Paquet et al., (2003) the AD group was divided into mild AD (n=12) and severe AD (n=14).

M Paired comparison tests between eyes within participants

Paired area	Mean	SD	t	p
Global RNFL (right eye – left eye)	2.311	8.325	1.862	.069
Temporal RNFL (right eye – left eye)	2.933	14.618	1.346	.185
Superior RNFL (right eye – left eye)	-2.900	14.360	-1.355	.182
Nasal RNFL (right eye – left eye)	1.800	14.490	.833	.409
Inferior RNFL (right eye – left eye)	7.011	20.295	2.317	.025
Fovea macular (right eye – left eye)	.129	7.535	.095	.925
Inner macular (right eye – left eye)	2.830	8.747	1.802	.082
Outer macular (right eye – left eye)	2.306	7.272	1.766	.088

Table M.1 Table showing no significant differences between retinal thickness scores dependent on eye

N Quality selection process for highest scan quality eye

The signal strength (0-40) of each scan was reviewed, and scans with signal strength of less than 15 (as suggested by the manufacturer) were excluded from the analysis. Nine eyes from the DS group had a rating of <15 and were therefore excluded, none of the control eye data fell below this threshold. For the macular scans the eye with the best quality scan from each participant was selected for analysis. In cases where only one eye had a full scan, this eye was chosen.

Macular scan quality index for the macular/posterior pole scan is assessed per each of the 61 individual line scans, therefore, in order to assess the average quality of the scan an average score was produced. As there was a large quantity of data to assess, approximately 176 scans each with 61 data entry points it was decided that a best call judgement would be made from scanning the data.

In the first instance 10 randomly selected participants were scanned and a best call judgement made, each quality index rating for each scan was then rated and average to provide the true result. Results were compared between the judgement call and the true result. The decision of the judgement call was also rated on difficulty, scored as “easy”, “medium” or “difficult” to determine. The same eye scans were independently inter-rated by a moderator Tables N.1 and N.2 show the results of the rating.

Correct decisions were made on 8/10 scans by the first examiner and 7/10 correct scans by the independent inter-rater, one decision was incorrectly made by both examiners. In all cases where an incorrect decision was made the difference between the quality indexes of the two scans was < 1.5. After all the best judgement decisions had been made a random nine participants were selected for re-analysis, of these three were incorrectly judged, however there was a negligible differences of < .37 between the quality index rating for each eye.

Group (ID number)	Actual best eye	Best eye decision (student)	Best eye decision (moderator)	Ease of decision (student)	Ease of decision (moderator)	Difference between eye quality average
Control (IDSC063)	Left	Left	Left	Easy	Easy	1.16
Control (IDSC003)	Right	Right	Right	Easy	Easy	4.97
Control (IDSC020)	Left	Left	Right	Medium	Difficult	1.18
Control (IDSC029)	Right	Left	Left	Difficult	Medium	.38
Control (IDSC030)	Left	Left	Left	Easy	Easy	1.57
DS (IDS036)	Right	Right	Right	Difficult	Easy	.56
DS (IDS407)	Left	Left	Left	Easy	Medium	4.49
DS (IDS021)	Right	Right	Right	Easy	Difficult	3.56
DS (IDS203)	Right	Left	Right	Difficult	Difficult	.92
DS (IDS039)	Left	Left	Right	Medium	Medium	1.49

Table N.1 Shows the best eye decision making process. The “actual best eye” column refers to the manual extraction of scan quality numbers and averaging to determine the highest value, the student and moderators ‘skimming decisions’ are highlighted as green for correct and blue for incorrect.

Group (code)	Actual best eye	Best eye decision (student)	Ease of decision	Difference between eye quality average
Control (IDSC041)	Left	Left	Difficult	.84
Control (IDSC021)	Right	Left	Difficult	.14
Control (IDSC072)	Left	Right	Medium	.36
Control (IDSC022)	Left	Right	Medium	.13
Control (IDSC046)	Left	Left	Easy	4.75
Control (IDSC013)	Left	Left	Easy	3.95
DS (IDS212)	Left	Left	Medium	.09
DS (IDS514)	Right	Right	Easy	.7
DS (IDS505)	Left	Left	Difficult	1.16

Table N.2 Showing retrospective checking of best eye decision made. 7/10 of decisions made were correct. In the cases of an incorrect decision being made the difference between the scan qualities of each eye was less than .36.

O Retinal layer age and thickness correlations for Down’s syndrome and age-matched control groups

Retinal layer	DS group	<i>p</i>	Control group	<i>p</i>
RNFL	-.087	.596	-.305	.066
GCL	-.220	.178	-.408	.012
IPL	-.118	.475	-.351	.033
INL	-.058	.727	-.217	.197
OPL	-.254	.118	.120	.480
ONL	.338	.035	-.236	.159
RPE	.160	.329	.311	.061

Table O.1 Table showing the non-significant correlations for the DS and age-matched control group retinal thicknesses and age

P CAMCOG neuropsychological assessment

SECTION 2 ASSESSMENT OF PATIENT/PARTICIPANT

The assessments in this section should be conducted with the patient/participant.

Part 1 Clinical interview

Each question should be asked as written. Additional questions may sometimes be necessary to clarify inadequate answers.

All items must be coded

Code:

Participant doesn't know (DK)

8 or 88

Not asked/not applicable (N/A)

9 or 99

158	Date of interview	<table border="1" style="margin: auto;"> <tr> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> </tr> </table>				

I would like to ask you some questions about how you are feeling.

DEPRESSION

159	How happy do you feel today:		Happy	0		Not very happy	1
	happy		No answer	8		Not happy at all	2
	not very happy		N/A	9			
	not happy at all?						
160	Do you feel sad or depressed?		No	0		Occasionally	1
			DK	8		Most of the time	2
			N/A	9			
161	Are there things that you used to enjoy doing that you don't enjoy any more?		No	0		Yes	1
			DK	8			
			N/A	9			
		<i>Record examples:</i>					

162	Have you lost your appetite or become much more hungry than usual?	No	0	Sometimes	1
		DK	8	Most of the time	2
		N/A	9		
<i>Specify:</i>					

CEREBROVASCULAR FUNCTION

163	Do you often have headaches?	No / rarely	0	Yes > 1 per week	1
		DK	8		
		N/A	9		
164	Do you often fall over?	No / rarely	0	Yes > 1 per month	1
		DK	8		
		N/A	9		

SLEEP

165	Do you find it difficult to fall asleep at night?	No	0	Yes	1
		DK	8		
		N/A	9		
166	Do you often wake up in the middle of the night?	No	0	Yes	1
		DK	8		
		N/A	9		
167	Do you often wake up too early in the morning and find it hard to go back to sleep?	No	0	Yes	1
		DK	8		
		N/A	9		

Now I would like to ask you some questions about any difficulties that you may have.

DECLINE IN FUNCTION

168	Are there things that you used to do that you find more difficult now?	No	0	Yes	1
		DK	8		
		N/A	9		
169	Do you find it more difficult to remember things than you used to?	No	0	Yes	1
		DK	8		
		N/A	9		
170	Do you forget where you have left things more than you used to?	No	0	Yes	1
		DK	8		
		N/A	9		
171	Do you forget the names of people you know well?	No	0	Yes	1
		DK	8		
		N/A	9		

Part 2 Cognitive Examination (CAMCOG-DS)

Neuropsychological assessment to be conducted with the patient/participant.

Before commencing, make sure you have the following items:

- | | | |
|-------------------------|----------|------------|
| Stimulus booklet | Pencil | Wristwatch |
| Blank sheet of A4 paper | Envelope | |

It is important that you speak slowly and clearly. If the participant appears not to have heard or understood, repeat the question (unless the item specifically prohibits repetition).

Do not give the correct answer if a wrong answer or no answer is given.

Coding: *Participants who don't know or refuse to give the answer or give a silly answer receive a score of 0 (equivalent to an incorrect answer). A score of 9 is recorded only if a question is not asked. In such cases indicate the reason for the omission of the question.*

'I am going to ask you some questions now to find out about your memory and other skills. Some of them may seem very easy and others may be difficult, but we need to ask everybody the same questions.'

ORIENTATION

172	What is your name?	First and surname	2
		First name (or surname) only	1
		Incorrect	0
		Not asked	9
173	What day is it today?	Correct without prompt	2
	<i>If no response ask:</i>	Correct with prompt	1
	Is it _____, _____ or _____?	Incorrect	0
	<i>(correct day of the week plus two others - correct answer 2nd)</i>	Not asked	9
174	What month is it now?	Correct without prompt	2
	<i>If no response ask:</i>	Correct with prompt	1
	Is it _____, _____ or _____?	Incorrect	0
	<i>(correct month plus two others - correct answer 1st)</i>	Not asked	9
175	What year is it now?	Correct without prompt	2
	<i>If no response ask:</i>	Correct with prompt	1
	Is it _____, _____ or _____?	Incorrect	0
	<i>(correct year plus two others - correct answer 3rd)</i>	Not asked	9

176	What is the name of this place? (<i>or if tested at home: What is this address?</i>)	Correct without prompt	2
		Correct with prompt	1
		Incorrect	0
		Not asked	9
	<i>If no response ask:</i> Is it _____, _____ or _____? (<i>correct place plus two alternatives – correct answer 2nd</i>)		
177	What is the name of this town (village, city)?	Correct without prompt	2
		Correct with prompt	1
		Incorrect	0
		Not asked	9
	<i>If no response ask:</i> Is it _____, _____ or _____? (<i>correct town plus two alternatives – correct answer 1st</i>)		

LANGUAGE

Comprehension

Motor Response			
<i>If the patient/participant does not complete the full sequence then the whole instruction may be repeated, without change in tone or tempo, to ensure that it has been heard and understood. Prompting and coaching stage by stage are not allowed. For questions 179-181 half marks are given for a partially correct sequence (e.g. left and right confused, only one of the required actions completed or actions completed in the wrong order)</i>			
I am going to ask you to do something, so please listen carefully.			
178	Please nod your head.	Correct	1
		Incorrect	0
		Not asked	9
179	Please touch your right ear with your left hand.	Correct	2
		Partially correct	1
		Incorrect	0
		Not asked	9
180	Please look at the ceiling and then look at the floor.	Correct	2
		Partially correct	1
		Incorrect	0
		Not asked	9
181	Please tap each shoulder twice with two fingers.	Correct	2
		Partially correct	1
		Incorrect	0
		Not asked	9

Expression

Naming

In questions 182 and 183 accurate naming is needed. Descriptions of function or approximate answers are not acceptable. Acceptable answers may depend on local usage. Some items may have more than one correct name (as indicated). Errors include description of function (e.g. 'used for telling time' for watch and approximate answers (e.g. 'weighing machine' for scales or 'light' for lamp).

In the case of approximate answers, you should say 'Can you think of another word for it?'

Tick each item correctly named and enter number correct under total.

182

Show pencil

What is this called?

Show wristwatch

What is this called?

Pencil

Watch

Total

Not asked

9

183

I am going to show you some pictures. Please tell me the name of each one.

Show pictures for naming in booklet.

Shoe

Computer

Scales

Suitcase

Clock

Lamp

Total

Not asked

9

Fluency

Only if the participant asks for clarification, explain that animals include birds, fish, insects etc. If the participant gets stuck, encourage him/her with 'Can you think of any more?'

Record number correct in one minute (repetitions not to be counted but age and sexual variants should be counted, e.g. calf, cow, bull).

184

I'd like you to tell me as many different animals as you can. See how many you can think of in one minute.

List all items:

Number correct

Recode for CAMCOG score

0

0

1-4

1

5-9

2

10-14

3

15+

4

Not asked

9

Definitions			
185	What do you do with a hammer?	Any correct use	1
		Incorrect	0
	<i>Hit is not enough. Some other detail should be given without prompting.</i>	Not asked	9
186	Where do people usually go to ^u by medicine?	Chemist / pharmacy	1
		Incorrect	0
		Not asked	9
187	What is a bridge?	Goes across river etc.	2
		Cross the bridge	1
	<i>A general (abstract) definition scores 2 and a specific or limited definition scores 1.</i>	Incorrect	0
		Not asked	9
Repetition			
188	I am going to say something and I'd like you to repeat it after me: 'People spend money'.	Correct	2
		Partially correct	1
		Incorrect	0
		Not asked	9

MEMORY

New Learning: Incidental Memory

Recall			
189	I showed you some pictures a little while ago. Can you remember what they were?	Shoe	<input type="checkbox"/>
		Computer	<input type="checkbox"/>
		Scales	<input type="checkbox"/>
		Suitcase	<input type="checkbox"/>
		Clock	<input type="checkbox"/>
		Lamp	<input type="checkbox"/>
		Total	[]
		Not asked	9
	<i>Either descriptions or names are acceptable. Tick each item correctly recalled and enter number correct under Total. If participant previously gave an incorrect name in question 183 but recalls it at this stage, score as correct.</i>		
Recognition			
190	Which one of these pictures did I show you before?	Shoe	<input type="checkbox"/>
		Computer	<input type="checkbox"/>
		Scales	<input type="checkbox"/>
		Suitcase	<input type="checkbox"/>
		Clock	<input type="checkbox"/>
		Lamp	<input type="checkbox"/>
		Total	[]
		Not asked	9

Retrieval of Remote Memories

<p><i>For questions 191 to 194, if the participant does not know the answer then give the clue provided. If the correct answer is given following the clue the participant scores 1.</i></p>			
191	Who was John Lennon?	One of the Beatles	2
		One of the Beatles (with clue)	1
	<i>Clue: He was in a famous pop group.</i>	Incorrect	0
		Not asked	9
192	Which Princess died in a car crash in Paris?	Princess Diana	2
		Princess Diana (with clue)	1
	<i>Clue: She was married to Prince Charles</i>	Incorrect	0
		Not asked	9

Retrieval of recent information

193	Who is the Prime Minister?	Correct	2
		Correct with clue	1
	<i>Clue: His/her first name is... (give first name)</i>	Incorrect	0
		Not asked	9
194	What is the name of the present king or queen?	Correct	2
		Correct with clue	1
	<i>Clue: It begins with... (give first letter)</i>	Incorrect	0
		Not asked	9

ATTENTION / CONCENTRATION

195	I would like you to count to twenty for me.	Highest number reached before error	[]
	<i>Record highest number reached before an error is made. Recode for CAMCOG score.</i>	<i>Recode (number reached before error)</i>	
		20	4
		15-19	3
		10-14	2
		5-9	1
		<5	0
		Not asked	9

196

Hold up 1st, 2nd and 3^d fingers: Look at my fingers. See, I'm holding up three fingers.
 Then hold up 1st finger: Now I'm holding up 1 finger.
 Then hold up 1st and 4th: Now you count my fingers. Yes, two fingers.
 Then hold up 1st finger only. If participant doesn't spontaneously count fingers say: I want you to count my fingers, keep counting, don't stop.

Tick each presentation that is counted spontaneously. Score as described.

1 st and 4 th	2	<input type="checkbox"/>
1 st	1	<input type="checkbox"/>
1 st 2 nd and 3 ^d	3	<input type="checkbox"/>
4 th	1	<input type="checkbox"/>
all 4 fingers	4	<input type="checkbox"/>

All correct (no prompt)	2
1 prompt	1
More than 1 prompt	0
Not asked	9

197

I'm going to say some numbers and I'd like you to repeat them after me...

Read each number string once. Tick each series correctly repeated. Discontinue after failure on both series of a given length. Score as described.

2	<input type="checkbox"/>
5	<input type="checkbox"/>
8-7	<input type="checkbox"/>
4-1	<input type="checkbox"/>
5-8-2	<input type="checkbox"/>
6-9-4	<input type="checkbox"/>
6-4-3-9	<input type="checkbox"/>
7-2-8-6	<input type="checkbox"/>
4-2-7-3-1	<input type="checkbox"/>
7-5-8-3-6	<input type="checkbox"/>

4 or 5 digit series correct	3
2 or 3 digit series correct	2
1 digit repeated	1
0 correct	0
Not asked	9

LANGUAGE

Comprehension

Reading				
<i>Show reading comprehension in booklet.</i>				
<i>It is not necessary for the participant to read aloud. If participant reads instruction but fails to carry out action, say 'now do what it says'</i>				
198	I would like you to read this and do what it says.	'Close your eyes'	Correct	1
			Incorrect	0
			Not asked	9
199	'Give me your hand'		Correct	1
			Incorrect	0
			Not asked	9

PRAXIS

Copying and drawing

<i>The participant should draw on the sheet of paper provided (p. 49).</i>				
200	Copy this shape (circle) <i>A closed circular shape (circle, oval or ellipse) is required</i>		Correct	1
			Incorrect	0
			Not asked	9
201	Copy this shape (square) <i>A closed four sided shape (square or rectangle) is required</i>		Correct	1
			Incorrect	0
			Not asked	9
202	Copy this picture (3D house) <i>Tick each component successfully completed and enter number under Total</i>		Outline of house	<input type="checkbox"/>
			Windows, doors and chimney in correct positions	<input type="checkbox"/>
			3D representation	<input type="checkbox"/>
			Total	[]
			Not asked	9
203	Draw a large clock and put all the numbers on it. <i>When participant has done this say 'Now set the hands to 10 past 11'.</i> <i>Tick each component successfully completed and enter number under Total</i>		Circle or square	<input type="checkbox"/>
			All numbers in correct position	<input type="checkbox"/>
			Correct time	<input type="checkbox"/>
			Total	[]
			Not asked	9

MEMORY

Registration

204	<p>Show picture of John Brown in booklet</p> <p>This is John Brown. Try to remember his name. <i>Short pause.</i></p> <p>What is his name?</p>	<p>Correct 2</p> <p>One name only 1</p> <p>Incorrect 0</p> <p>Not asked 9</p>
	<p><i>If incorrect or partially correct say 'his name is John Brown'</i></p>	
205	<p>I'm going to tell you where he lives. See if you can remember.</p> <p>He lives at:</p> <p>42 West Street, Bedford. <i>Short pause</i></p> <p>Where does he live?</p>	<p>Correct 2</p> <p>Partially Correct 1</p> <p>Incorrect 0</p> <p>Not asked 9</p>
	<p><i>If incorrect or partially correct say 'he lives at 42 West Street, Bedford'</i></p> <p>Please try to remember his name and address as I will be asking you about them later on.</p>	

PRAXIS

Ideomotor

<p><i>For questions 206 to 208, a correct mime is needed. If the participant uses fingers to represent scissors or brush, say e.g. 'Pretend you are holding a toothbrush'. Score 1 if brushing movement is made but not as though holding a toothbrush.</i></p>		
206	<p>Show me how you wave goodbye.</p>	<p>Correct 1</p> <p>Incorrect 0</p> <p>Not asked 9</p>
	<p>Show me how you would cut with scissors.</p>	<p>Correct 2</p> <p>Partially Correct 1</p> <p>Incorrect 0</p> <p>Not asked 9</p>
208	<p>Show me how you would brush your teeth with a toothbrush.</p>	<p>Correct 2</p> <p>Partially Correct 1</p> <p>Incorrect 0</p> <p>Not asked 9</p>

Ideational

<p><i>Read the following statement and then hand a sheet of paper to the participant. Make a point of handing to the participant's midline. No repetition of this question is allowed. Speak slowly and clearly, having first made sure you have the participant's full attention.</i></p>			
209	<p>I am going to give you a piece of paper. When I do, take the paper in your right hand. Fold the paper in half with both hands, and put the paper down on your lap.</p> <p><i>Do not repeat instructions or coach. Score a move as correct only if it takes place in the correct sequence. Tick each correct move and enter number correct under total.</i></p>	Right hand	<input type="checkbox"/>
		Folds	<input type="checkbox"/>
		On lap	<input type="checkbox"/>
		Total	[1
		Not asked	9
210	<p><i>Hand envelope to participant</i></p> <p>Put the paper in the envelope and seal the envelope.</p>	Correct	1
		Incorrect	0
		Not asked	9
		Not asked	9

MEMORY

Intentional Learning

<p>Recall</p>					
211	<p><i>Show picture of John Brown in booklet</i></p> <p>What was this man's name?</p>	Full name correct	2		
		Partially correct	1		
		Incorrect	0		
		Not asked	9		
212	<p>What was his address?</p> <p><i>Tick each item recalled correctly and enter number correct under Total</i></p>	42	<input type="checkbox"/>		
		West Street	<input type="checkbox"/>		
		Bedford	<input type="checkbox"/>		
				Total	[]
				Not asked	9

ABSTRACT THINKING

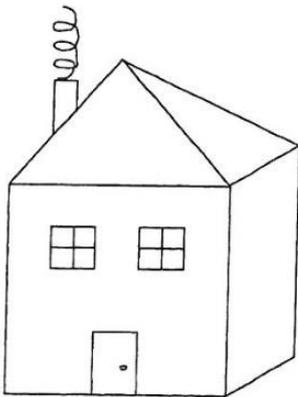
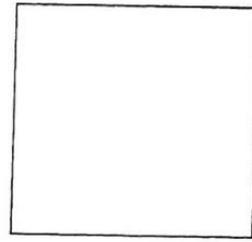
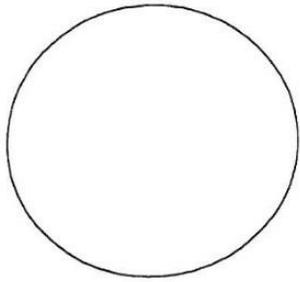
These questions investigate the capacity to work out general relationships between objects. Fully correct answers score 2. Partially correct answers score 1. Examples are given beside each score if the participant says 'They are not alike', say 'They are alike in some way. Can you tell me in which way are they alike?'

I am going to name two things and I would like you to tell me in what way they are alike. For example a dog and a monkey are alike because they are both animals.

213	In what way are an apple and a banana alike?	Fruit	2
		Food, grow, have peel	1
		Round, have calories	0
		Not asked	9
	<i>Record answer</i>		
	<i>For this question only, if incorrect say 'they are also alike because they are both kinds of fruit'.</i>		
214	In what way are a shirt and a dress alike?	Clothing	2
		To wear, made of cloth, keep warm	1
		Have buttons	0
		Not asked	9
	<i>Record answer</i>		
215	In what way are a table and a chair alike?	Furniture	2
		Household objects, used for meals	1
		Wooden, 4 legs	0
		Not asked	9
	<i>Record answer</i>		

VISUAL PERCEPTION

216	<i>Show 'Recognition of famous people' in booklet</i>	Queen	<input type="checkbox"/>
	Who is this?	Pope, Archbishop, Bishop	<input type="checkbox"/>
	<i>Score as correct if picture is recognised.</i>	Total	[]
	<i>Correct name is not required, but record any answer that does not correspond exactly to the examples given.</i>	Not asked	9
217	<i>Show 'Recognition of objects' in booklet</i>	Spectacles	<input type="checkbox"/>
	These pictures are taken from unusual angles.	Shoe	<input type="checkbox"/>
	Can you tell me what they are?	Purse/suitcase	<input type="checkbox"/>
	<i>Criterion is whether object is recognised but not that it is named correctly, therefore descriptions of function are acceptable. Tick each item answered correctly and enter number correct under Total</i>	Cup and saucer	<input type="checkbox"/>
		Telephone	<input type="checkbox"/>
		Pipe	<input type="checkbox"/>
		Total	[]
	Not asked	9	



Clock

CAMCOG-DS Scoring Summary Chart

Participant's Name _____ Assessment Date ___/___/___

<p>ORIENTATION Max</p> <p>172 Name <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>173 Day <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>174 Month <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>175 Year <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>176 Address <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>177 Town <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>Orientation Total <input style="width: 30px; height: 20px; background-color: #cccccc;" type="text"/> (12)</p>	<p>MEMORY Max</p> <p>New Learning</p> <p>189 Recall pictures <input style="width: 30px; height: 20px;" type="text"/> (6)</p> <p>190 Recognition <input style="width: 30px; height: 20px;" type="text"/> (6)</p> <p>204 Register name <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>205 Register address <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>211 Recall name <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>212 Recall address <input style="width: 30px; height: 20px;" type="text"/> (3)</p> <p>Total <input style="width: 30px; height: 20px;" type="text"/> (21)</p> <p>Remote</p> <p>191 Lennon <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>192 Diana <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>Total <input style="width: 30px; height: 20px;" type="text"/> (4)</p> <p>Recent</p> <p>193 Prime Minister <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>194 Monarch <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>Total <input style="width: 30px; height: 20px;" type="text"/> (4)</p> <p>Memory Total <input style="width: 30px; height: 20px; background-color: #cccccc;" type="text"/> (29)</p>	<p>PRAXIS Max</p> <p>Drawing/copying</p> <p>200 Circle <input style="width: 30px; height: 20px;" type="text"/> (1)</p> <p>201 Square <input style="width: 30px; height: 20px;" type="text"/> (1)</p> <p>202 House <input style="width: 30px; height: 20px;" type="text"/> (3)</p> <p>203 Clock <input style="width: 30px; height: 20px;" type="text"/> (3)</p> <p>Total <input style="width: 30px; height: 20px;" type="text"/> (8)</p> <p>Actions to command</p> <p>206 Wave <input style="width: 30px; height: 20px;" type="text"/> (1)</p> <p>207 Scissors <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>208 Toothbrush <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>209 Paper <input style="width: 30px; height: 20px;" type="text"/> (3)</p> <p>210 Envelope <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>Total <input style="width: 30px; height: 20px;" type="text"/> (10)</p> <p>Praxis Total <input style="width: 30px; height: 20px; background-color: #cccccc;" type="text"/> (18)</p>
<p>LANGUAGE</p> <p>Comprehension</p> <p>178 Nod <input style="width: 30px; height: 20px;" type="text"/> (1)</p> <p>179 Ear <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>180 Ceiling <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>181 Shoulder <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>198 Eyes <input style="width: 30px; height: 20px;" type="text"/> (1)</p> <p>199 Hand <input style="width: 30px; height: 20px;" type="text"/> (1)</p> <p>Total <input style="width: 30px; height: 20px;" type="text"/> (9)</p> <p>Expression</p> <p>182 Objects <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>183 Pictures <input style="width: 30px; height: 20px;" type="text"/> (6)</p> <p>184 Fluency <input style="width: 30px; height: 20px;" type="text"/> (4)</p> <p>185 Hammer <input style="width: 30px; height: 20px;" type="text"/> (1)</p> <p>186 Chemist <input style="width: 30px; height: 20px;" type="text"/> (1)</p> <p>187 Bridge <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>188 Repetition <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>Total <input style="width: 30px; height: 20px;" type="text"/> (18)</p> <p>Language Total <input style="width: 30px; height: 20px; background-color: #cccccc;" type="text"/> (27)</p>	<p>ATTENTION</p> <p>195 Twenty <input style="width: 30px; height: 20px;" type="text"/> (4)</p> <p>196 Fingers <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>197 Digit-span <input style="width: 30px; height: 20px;" type="text"/> (3)</p> <p>Attention Total <input style="width: 30px; height: 20px; background-color: #cccccc;" type="text"/> (9)</p>	<p>ABSTRACT THINKING</p> <p>213 Fruit <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>214 Clothing <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>215 Furniture <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>Abstr. Thinking Total <input style="width: 30px; height: 20px; background-color: #cccccc;" type="text"/> (6)</p>
		<p>PERCEPTION</p> <p>216 People <input style="width: 30px; height: 20px;" type="text"/> (2)</p> <p>217 Unusual views <input style="width: 30px; height: 20px;" type="text"/> (6)</p> <p>Total <input style="width: 30px; height: 20px; background-color: #cccccc;" type="text"/> (8)</p>
		<p>TOTAL SCORE <input style="width: 30px; height: 20px; background-color: #cccccc;" type="text"/> (109)</p>

Part 3 Interviewer Observations

To be recorded at the end of the patient/participant assessment.
Code 'Yes' only if characteristic is markedly present.

218	Self-neglect	No	0	Yes	1
219	Uncooperative behaviour	No	0	Yes	1
220	Suspiciousness	No	0	Yes	1
221	Hostility or irritability: e.g. angry response	No	0	Yes	1
222	Silly, incongruent or bizarre behaviour	No	0	Yes	1
223	Slowness and underactivity: e.g. sits abnormally still, delay in response to questions.	No	0	Yes	1
224	Restlessness: e.g. fidgeting, pacing, unnecessary movements.	No	0	Yes	1
225	Anxiety and fear: appears frightened, worried or somatically tense out of proportion to the situation.	No	0	Yes	1
226	Depressed mood: looks sad, mournful, tearful, voice low or gloomy	No	0	Yes	1
227	Lability of mood: rapidly changes from sad to happy, friendly to irritable.	No	0	Yes	1
228	Flat affect: lack of spontaneous emotion or emotional response to interviewer, monotonous voice, lack of gestures	No	0	Yes	1
229	Hallucinating: behaves as though hears voices or sees visions, or admits to doing so.	No	0	Yes	1
230	Speech very rapid and difficult to follow.	No	0	Yes	1
231	Speech very slow with pauses between words.	No	0	Yes	1
232	Speech restricted in quantity: e.g. answers questions but no spontaneous expressions.	No	0	Yes	1

233	Speech rambling or incoherent, irrelevant answers to questions.	No	0	Yes	1
234	Speech slurred.	No	0	Yes	1
235	Perseveration.	No	0	Yes	1
236	Clouding of consciousness.	No	0	Yes	1
237	Speaks to self	No	0	Yes	1
238	Impaired ability to focus, sustain or shift attention.	No	0	Yes	1
239	Hypochondriacal preoccupations with somatic discomfort.	No	0	Yes	1

Q Executive function battery

Imaging Brain A β in Adults with Down's Syndrome

Neuropsychological Test Battery - REVISED

Participant Number:.....

Date of Assesment:.....

Researcher's name:

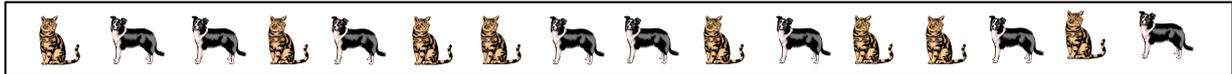
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<u>Page</u>	<u>Test Name</u>
3	Cats and Dogs
5	Tower of London
7	Wiegl Sorting Task
9	Spatial Reversal
11	Scrambled Boxes
13	Oliver Object Memory Test
14	Oliver memory for Sentences

Cats and Dogs

This test was adapted by Sarah Ball (2006) from Willner et al.'s Day and Night test. Ball's version of the test involves participants pointing to a series of animals and naming them.

A sequence of 16 pictures, 8 cats (c) and 8 dogs (d) was presented on a single strip in the following order: c,d,d,c,d,c,c,d,d,c,d,c,c,d,c,d



Say, **“Now I am going to ask you to name some pictures of animals. I want you to point to each picture, and tell me the name of the animal that you see. I want you to do this as quickly as you can. I’ll have a go first to show you.”**

Demonstrate what is required of the participant by pointing to each animal in turn and naming it.

After you have demonstrated this, the participant should have a practice go. Say, **“Now you have a go, have a practice.”** If they get the first FOUR animals correct, stop them and say. **“That’s it, you’ve got the hang of it.”**

Then say, **“Ok let’s have a proper go now. Remember, I want you to name each animal as quickly as you can. Are you ready? Ok, go!”**

When the participant has finished, say, **“Well done, that was very good.”**

In the next part of the task, participants must again point to each picture in turn, but this time if they point to a cat they must say ‘dog’, and if they point to a dog they must say, ‘cat’.

Say to the participant, **“Ok, now we’re going to change the rule. This time, if you point at a cat you must say ‘Dog’, and if you point to a dog you must say ‘Cat’. Ok? Can you remember that? I’ll have a go first to show you”**

Demonstrate what is required of the participant by pointing to each animal in turn and naming it according to the new rule.

After you have demonstrated this, the participant should have a practice go. Say, “**Now you have a go, have a practice.**” If they get the first FOUR animals correct, stop them and say, “**That’s it, you’ve got the hang of it.**”

Then say, “**Ok let’s have a proper go now. Remember, I want you to name each animal as quickly as you can, but try and remember the new rule. Are you ready? Ok, go!**”

When the participant has finished, say, “**Well done, that was very good.**”

Cats and Dogs Task

Name.....

Subject Number.....

SCORE REVERSAL

<u>Naming</u>	<u>Switching response</u>
CAT <input type="checkbox"/>	DOG <input type="checkbox"/>
DOG <input type="checkbox"/>	CAT <input type="checkbox"/>
DOG <input type="checkbox"/>	CAT <input type="checkbox"/>
CAT <input type="checkbox"/>	DOG <input type="checkbox"/>
DOG <input type="checkbox"/>	CAT <input type="checkbox"/>
CAT <input type="checkbox"/>	DOG <input type="checkbox"/>
CAT <input type="checkbox"/>	DOG <input type="checkbox"/>
DOG <input type="checkbox"/>	CAT <input type="checkbox"/>
DOG <input type="checkbox"/>	CAT <input type="checkbox"/>
CAT <input type="checkbox"/>	DOG <input type="checkbox"/>
DOG <input type="checkbox"/>	CAT <input type="checkbox"/>
CAT <input type="checkbox"/>	DOG <input type="checkbox"/>
CAT <input type="checkbox"/>	DOG <input type="checkbox"/>
DOG <input type="checkbox"/>	CAT <input type="checkbox"/>
CAT <input type="checkbox"/>	DOG <input type="checkbox"/>
DOG <input type="checkbox"/>	CAT <input type="checkbox"/>
Time taken <input type="text"/> secs	Time taken <input type="text"/> secs
Errors <input type="text"/>	Errors <input type="text"/>

Difference in time taken
 Difference in errors made

Score: 16 - errors at reversal stage = points

Tower of London Task

Procedure adapted from Kirkorian, Bartok & Gay, 1994, *J Clin Exptl Neuropsychology*, 16, 6, 840-850.

Sit opposite the participant and place the two boards with the longest peg on the participant's left and the numbers 1, 2, 3 pointing towards you; one in front of them, with the beads in the start position, and one in front of you, with the beads in the practice trial position (Practice 1).

Say **"I want you to move the beads on your pegs to make them the same as the beads on my pegs."**

When they have done it say, **"that's good. That's the idea. Each time we'll start with your beads like this** (arrange the beads into the start position). **I'll change mine and you have to make yours the same as mine. I also want you to do it in a certain number of moves. A move means taking a bead from a peg and putting it on another peg. There are two rules. You can only pick up one bead at a time so you can't do this** (pick up two beads in one hand) **or this** (pick up one bead in each hand). **You can't put the beads down on the table. If you get stuck we can go back to the beginning. Shall we have a practice?"**

Arrange your board to the first practice problem again. Say, **"Now, try and make yours look like this in two moves."**

Provide feedback if the participant tries to make an illegal move and about the number of moves allowed. Say, **"Remember the rule. You can't..... . Let's start again. Have another go. Remember that you have to do it in (X) moves."**

Provide one more practice trial with feedback as above.

After practice, go through the problems in the order presented on the sheet and continue to provide feedback if the participant tries to make an illegal move and about the number of moves allowed for the problem. Say, **"Remember the rule. You can't..... . Let's start again. Have another go. Try again. Remember that you have to do it in (X) moves."**

Discontinue after three consecutive failed problems (i.e. 9 consecutive failed trials).

Scoring

The pegs are labelled 1, 2, 3 (small to large) and the beads are called R, G, B. Write down the moves the participant makes on the score sheet. The problem is solved if the end position is achieved in the required number of legal moves.

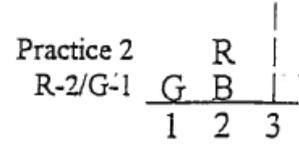
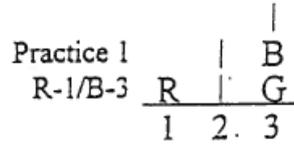
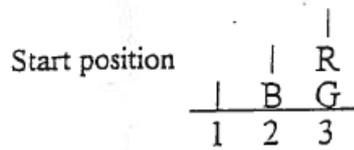
A trial ends if the participant realises that the trial will not succeed or if an illegal move is made. The beads are then reset to the start position for the next trial. Do not provide feedback after each incorrect move, wait until the end of the trial.

A participant is allowed to modify a move if they are still holding a bead.

Three trials will be allowed for each problem. Three points are scored if the problem is solved on the first trial, two if on the second trial, and one point if on the third.

Tower of London Task – Record Sheet

Name..... Subj. number..... Assessor.....



Trial	Target	# moves	Correct response	Subject response	Score												
1	<table style="border-collapse: collapse; margin: auto;"> <tr> <td style="border-right: 1px solid black; padding: 0 5px;"></td> <td style="border-right: 1px solid black; padding: 0 5px;"></td> <td style="border-right: 1px solid black; padding: 0 5px; text-align: center;">R</td> </tr> <tr> <td style="border-right: 1px solid black; padding: 0 5px; text-align: center;">B</td> <td style="border-right: 1px solid black; padding: 0 5px; text-align: center;">R</td> <td style="padding: 0 5px; text-align: center;">G</td> </tr> </table>			R	B	R	G	2	B-1/R-2	<table style="width: 100%; border-collapse: collapse;"> <tr><td style="border-bottom: 1px solid black; height: 15px;"></td></tr> <tr><td style="border-bottom: 1px solid black; height: 15px;"></td></tr> <tr><td style="border-bottom: 1px solid black; height: 15px;"></td></tr> </table>				<table style="width: 100%; border-collapse: collapse;"> <tr><td style="border-bottom: 1px solid black; height: 15px; text-align: center;">3</td></tr> <tr><td style="border-bottom: 1px solid black; height: 15px; text-align: center;">2</td></tr> <tr><td style="border-bottom: 1px solid black; height: 15px; text-align: center;">1</td></tr> </table>	3	2	1
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4	<table style="border-collapse: collapse; margin: auto;"> <tr> <td style="border-right: 1px solid black; padding: 0 5px;"></td> <td style="border-right: 1px solid black; padding: 0 5px; text-align: center;">G</td> <td style="border-right: 1px solid black; padding: 0 5px;"></td> </tr> <tr> <td style="border-right: 1px solid black; padding: 0 5px;"></td> <td style="border-right: 1px solid black; padding: 0 5px; text-align: center;">R</td> <td style="padding: 0 5px; text-align: center;">B</td> </tr> </table>		G			R	B	5	B-1/R-2/G-2/B-3/ G-3	<table style="width: 100%; border-collapse: collapse;"> <tr><td style="border-bottom: 1px solid black; height: 15px;"></td></tr> <tr><td style="border-bottom: 1px solid black; height: 15px;"></td></tr> <tr><td style="border-bottom: 1px solid black; height: 15px;"></td></tr> </table>				<table style="width: 100%; border-collapse: collapse;"> <tr><td style="border-bottom: 1px solid black; height: 15px; text-align: center;">3</td></tr> <tr><td style="border-bottom: 1px solid black; height: 15px; text-align: center;">2</td></tr> <tr><td style="border-bottom: 1px solid black; height: 15px; text-align: center;">1</td></tr> </table>	3	2	1
	G																
	R	B															
3																	
2																	
1																	
Total score																	

The Weigl Sorting Test

This is a version of Weigl’s test (aka Weigl-Goldstein-Scheerer Colour Form Sorting Test; Goldstein & Scheerer, 1941, 1953; Weigl, 1941) administered and scored in a similar way to Strauss & Lewin, 1982.

It consists of 12 blocks of four colours (red, green, blue, yellow) and three shapes (circle, square, triangle).

Name..... Subj. Number.....

Assessor.....

Spread out the block in front of the participant.

Say, “**Here are some blocks. I want you to sort them so that the ones which belong together will be in a pile together.**”

If the participant is finding the initial sort difficult, repeat the instructions or prompt, “**Put the ones which are similar in a pile together.**”

Initial Sort achieved? No OR Yes According to Colour/Shape (circle appropriate).

Say, “**Why have you grouped them in this way? (In case of difficulty, prompt with) How do these ones belong together? So why have you put them in these piles?**”

Response.....
.....

Say, “**Now I want you to sort the blocks so that they belong together in a different way.**”

Shift achieved now? No OR Yes Level 1

If shift is not achieved, say, “**Here you have grouped them according to Colour/Shape. This time you must group the differently.**”

Shift achieved now? No OR Yes Level 2

If shift is not achieved, say, “**Here you have put them in piles of Red, Green, Blue & Yellow/Triangular, Square and Round. This time try to make the piles differently.**”

Shift achieved now? No OR Yes Level 3

Allow up to a total of four trials, for the fifth trial sort three blocks according to the new rule. Then say, “**I’ve started it off. See if you can put the rest of the blocks in piles like this.**”

Shift achieved now? No OR Yes Level 4

If shift is not achieved give the new rule explicitly. Say, “**can you put them in piles of different shapes/colours?**”

Shift achieved now? No OR Yes Level 5

After a shift is achieved at any level 1-4, ask “**Why have you grouped them in this way? (In case of difficulty, prompt with) How do these ones belong together? So why have you put them in these piles?**”

Response.....

Scoring

Failed to sort initially

Failed to shift at level **0 Points**

Shifted

Please encircle:

At level	1	2	3	4	5
Points awarded	5	4	3	2	1

Spatial Reversal

This test is a modified version of that by McEvoy et al. (1993), adapted by Sarah Ball for use with participants with intellectual disabilities.

The Set-Attainment Stage

In this stage of the task, the participant is instructed to find a coin hidden underneath one of two identical boxes, placed either side of the participant's midline. While the coin was being hidden, the boxes should be out of the participant's view behind a cardboard screen.

Put up the cardboard screen and hide a coin underneath *BOTH* boxes to ensure that the participant's search is successful. When the coins are hidden, remove the screen.

Say to the participant, "**Ok, there is a coin hidden underneath one of the boxes. See if you can find it.**"

Once the participant has selected a box and revealed the coin, congratulate them on doing well. Then replace the screen, removing the coin that was not found. Then place the coin that was found back underneath the box they first searched in.

Say, "**There is a rule about which side the coin will be hidden. See if you can work out where it will be this time.**"

If the participant selects the correct box say, "**Well done! You've got the hang of this. Let's have another go.**"

If the participant picks the empty box say, "**Never mine! Let's have another go. See if you can work out what the rule is.**" Continue to hide the coin underneath the box on the same side until the participant has made four consecutive correct responses, at which point, go to '*The Reversal Stag.*'

If four consecutive correct responses are NOT made, continue until the coin has been hidden on the same side for ten trials (at which point, the session should end).

The Reversal Stage

In this stage the rule is reversed **WITHOUT TELLING THE PARTICIPANT**. The procedure is repeated with the coin hidden on the opposite side. The aim of this stage is to measure how many trials it takes before the participants adjust their responding pattern according to the new rule. The session should be stopped after four consecutive correct responses, or after ten trials.

Remember: Put up the cardboard screen when hiding the coin on each trial. Congratulate participants on correct responses, and encourage them to try again when they make mistakes.

Spatial Reversal Score Sheet

Spatial reversal training					
Set attainment			Reversal		
Site of hiding L / R			Site of hiding L / R		
Trial	Correct (tick)	Score	Trial	Correct (tick)	Score
1			1		
2			2		
3			3		
4			4		7
5			5		6
6			6		5
7			7		4
8			8		3
9			9		2
10			10		1
4 consecutive correct?		Yes	4 consecutive correct?		Yes
		No 0			No 0
			Score		

Scrambled Boxes

Equipment: Small boxes with shapes on. Three coins.

Stationary condition

Say to the participant, "I am going to put a coin under each of these boxes". Then *hide the coins*. After a short delay, say, "I would like you to choose one of the boxes and look under it to see if you can find a coin."

After the participant has found a coin, remove it and say, "Now lets put that box back and try to find another coin." After replacing the empty box in its original position, say, **"This time I would like you to choose a box you haven't looked in before. Try not to choose an empty one."**

If participant successfully chooses a full box then say **"Well done! Now try again and see if you can find another coin. Try to choose a box you haven't looked under before"**

If participant chooses the empty box then say **"Oh dear it's empty. Never mind, let's have another go. Try to choose a box you haven't looked in before"**

Repeat as above until participant has found all the coins or has made FOUR of errors.

If participant successfully finds all coins then say **"Well done, you've found all the coins now. Let's try the same thing again but this time, to make it a bit more difficult, I'm going to move the boxes around."**

Scrambled condition

Say, **"I am going to put a coin under each of the boxes again"**. Then *hide the coins* .

After a short delay, say, **"Now I'm going to mix them up. I would like you to choose one of the boxes and look under it to see if you can find a coin."** *While giving this instruction mix up the location of the boxes while the participant watches.*

When the participant finds a coin, say "Now let's put that box back and try to find another coin."

Replace empty box and after a short delay, say to them, "I'm going to mix the boxes up again and I would like you to choose a box you haven't looked in before. Try not to choose an empty one." *While giving this instruction mix up the location of the boxes again while the participant watches.*

If participant successfully chooses a full box then say, **"Well done! Now try again and see if you can find another coin. Try to choose a box you haven't looked under before."** *Again, while giving this instruction mix up the location of the boxes while the participant watches.*

If participant chooses the empty box then say **"Oh dear it's empty. Never mind, let's have another go. Try to choose a box you haven't looked in before"** *Again, while giving this instruction mix up the location of the boxes while the participant watches.*

Continue until participant has found all the coins or made FOUR of errors.

If the participant completes both the stationary and scrambled stages with three boxes, then repeat the process with SIX boxes. In this case, testing continues until all the coins have been retrieved or until SEVEN errors have been made.

Boxes Working Memory Task					
Name					
Subject Number					
3 boxes stationary					
					
<input type="checkbox"/>	<input type="checkbox"/>				
6 boxes stationary					
					
<input type="checkbox"/>	<input type="checkbox"/>				
SCORE THE SCRAMBLED STAGE					
3 boxes scrambled					
					
<input type="checkbox"/>	<input type="checkbox"/>				
Score = 4 - errors					<input type="checkbox"/>
6 boxes scrambled					
					
<input type="checkbox"/>	<input type="checkbox"/>				
Score = 7 - errors					<input type="checkbox"/>
Total score: /11					

Oliver Object N

This test was first developed by Oliver et al. (1998) and was designed to measure memory deficits in older people with Down's syndrome.

Equipment needed: Spoon, letter (addressed envelope with stamp), pencil, key, comb, toothbrush, watch, coin (10p), purse and a book.

Training Stage

First say to the participant, "**I want you to name some objects for me now.**"

Show the participant one object at a time, asking "**What is this?**" each time you show them an object. Discard any objects they name incorrectly.

Then, take two objects that they participant named correctly. Ask again, "**What is this?**" for each object. Then say, "**Now I am going to cover of these,**" and while the participant watches, cover one of the objects with a small box. Then say to the participant, "**What's under the box?**"

Testing Stage

Randomly select two other correctly named objects and ask the participant to name them (to ensure they have looked at them). Then say, "**Try to remember these. Now close your eyes.**" While the participant's eyes are closed, cover one of the objects with the box and record the hidden stimulus on the record sheet. Then say, "**Open your eyes. What's under the box?**" Make a record of the answer.

Repeat this process once more with **2** objects. Then do the same twice with **3** objects, twice with **4** objects, twice with **5** objects and twice with **6** objects. Testing should stop if the participant makes an error on both tasks with the same number of objects.

Scoring

One point should be awarded for each object correctly recalled. The maximum score is 10.

Number of Objects	Stimulus Object	Response	Score
2			
2			
3			
3			
4			
4			
5			
5			
6			
6			
Total Score (max 10)			

The Oliver Memory for Sentences Test

This test was developed by Crayton and Oliver (1993) for use with older adults with DS. It was adapted from the Stanford-Binet test.

Training Stage

Say to the participant, “**I am going to say something and I want you to say it after me. Say ‘Watch’.**” Then wait for a response. Then say, “**Say ‘coin’.**” After giving them each word, wait for their response. Repeat this process for all of the objects in the Oliver Object Memory Test until the participant repeats the word correctly. Immediately after the training stage, move into the testing stage.

Testing Stage

Say to the participant, “**Say ‘He had a book’.**” Then write down what the participant says on the score sheet below. Do this for each of the six sentences.

Scoring

Give one point for each word correctly recalled.

Sentences		Score /max
Test	He had a book	
Response		/4
Test	His father saw him leave	
Response		/5
Test	They took their water from the tap	
Response		/7
Test	She went to the table to eat her food	
Response		/9
Test	My brother does not like me to sleep in his room	
Response		/11

Test	Each week they find a pretty flower and plant it in the garden	
Response		/13
Total Score (max 49)		

Scoring

Executive Functioning	
Tower of London	/12
Wiegl Sorting Task	/5
Spatial Reversal	/7
Scrambled Boxes	/11
Cats and Dogs	/16
TOTAL	/56

Memory		/156
Oliver Memory for Object	/10	
Oliver Memory for Sentences	/49	
TOTAL	/100	

GRAND TOTAL

R CAMDEX

BACKGROUND INFORMATION

Patient / Participant Details

1	Name	<input type="text"/>	
2	Address	<input type="text"/>	
3	Referral source	Self	1
		Relative	2
		Carer	3
		GP	4
		Inpatient Service	5
		Consultant / Specialist	6
		Other (specify)	7
4	Reason for Assessment	Clinical	1
		Research	2
5	Date of Birth	<input type="text"/>	<input type="text"/>
6	Age at Assessment	<input type="text"/>	
7	Sex	Male	1
		Female	2
8	Current marital status	Single	1
		Married	2
		Widowed	3
		Divorced	4
		Cohabiting	5
9	Living arrangements	Long-term hospital	1
		Nursing home	2
		Residential home	3
		Sheltered accommodation	4
		Supported Living	5
		Home with relative	6
		Own home with partner	7
		Own home alone	8
		Other	9

SECTION 1 INFORMANT INTERVIEW

This interview should be conducted with a relative, friend or carer who has known the patient / participant for at least 6 months, in order that they are able to comment on changes in functional ability or behaviour over time.

Each question should be asked as written. Additional questions may sometimes be necessary to clarify inadequate answers.

All items must be coded

Code:

Informant doesn't know (DK)

8 or 88

Not asked/not applicable (N/A)

9 or 99

Informant Details

10	Date of interview	<input style="width: 100%; height: 20px;" type="text"/>														
11	Informant's name	<input style="width: 95%; height: 20px;" type="text"/>														
12	Informant's address	<input style="width: 95%; height: 40px;" type="text"/>														
13	How was the interview conducted?	<table style="width: 100%; border: none;"> <tr> <td style="width: 60%;">Face to face</td> <td style="width: 5%; text-align: center;">1</td> </tr> <tr> <td>Telephone</td> <td style="text-align: center;">2</td> </tr> </table>	Face to face	1	Telephone	2										
Face to face	1															
Telephone	2															
14	What is your relationship to _____ ?	<table style="width: 100%; border: none;"> <tr> <td style="width: 60%;">Parent</td> <td style="width: 5%; text-align: center;">1</td> </tr> <tr> <td>Sibling</td> <td style="text-align: center;">2</td> </tr> <tr> <td>Spouse</td> <td style="text-align: center;">3</td> </tr> <tr> <td>Other relative</td> <td style="text-align: center;">4</td> </tr> <tr> <td>Friend</td> <td style="text-align: center;">5</td> </tr> <tr> <td>Carer/keyworker</td> <td style="text-align: center;">6</td> </tr> <tr> <td>Other (specify)</td> <td style="text-align: center;">7</td> </tr> </table>	Parent	1	Sibling	2	Spouse	3	Other relative	4	Friend	5	Carer/keyworker	6	Other (specify)	7
Parent	1															
Sibling	2															
Spouse	3															
Other relative	4															
Friend	5															
Carer/keyworker	6															
Other (specify)	7															
15	How long have you known him/her?	<input style="width: 30px;" type="text"/> Years <input style="width: 30px;" type="text"/> Months														
16	How often do you see him/her?	<table style="width: 100%; border: none;"> <tr> <td style="width: 60%;">Lives with</td> <td style="width: 5%; text-align: center;">1</td> </tr> <tr> <td>Daily</td> <td style="text-align: center;">2</td> </tr> <tr> <td>More than once a week</td> <td style="text-align: center;">3</td> </tr> <tr> <td>At least once a week</td> <td style="text-align: center;">4</td> </tr> <tr> <td>At least once a month</td> <td style="text-align: center;">5</td> </tr> <tr> <td>At least once a year</td> <td style="text-align: center;">6</td> </tr> </table>	Lives with	1	Daily	2	More than once a week	3	At least once a week	4	At least once a month	5	At least once a year	6		
Lives with	1															
Daily	2															
More than once a week	3															
At least once a week	4															
At least once a month	5															
At least once a year	6															

Part 1 Patient/participant's Best Level of Functioning

Instructions to be read to informant:

"The aim of the next set of questions I am going to ask, is to find out abouts abilities when he/she was functioning **at his/her best** (before any decline in function). If there has been no decline, this will be as he/she is functioning now."

A EDUCATION AND EMPLOYMENT

17	Has he/she ever attended school?	No	0	Yes, special school	1
		DK	8	Yes, special class in mainstream	2
		N/A	9	Yes, mainstream	3
18	If 'yes', for how long did she attend?	DK	88	____ ____ Years	____ ____ Months
		N/A	99		
19	Has he/she ever attended college or day centre?	No	0	Yes	1
		DK	8		
		N/A	9		
20	If 'yes', for how long did she attend?	DK	88	____ ____ Years	____ ____ Months
		N/A	99		
21	Has he/she ever had a job?	No	0	Yes	1
		DK	8	<i>If 'yes', give details:</i>	
		N/A	9		

B BASIC SKILLS

22	Has he/she ever been able to speak?	No	0	Yes, single words only	1
		DK	8	Yes, limited speech	2
		N/A	9	Yes, good speech	3
23	Has he/she ever been able to understand spoken language?	No	0	Yes, single words only	1
		DK	8	Yes, limited understanding	2
		N/A	9	Yes, good understanding	3
24	Has he/she ever been able to read?	No	0	Yes, a little, familiar words	1
		DK	8	Yes, good reader	2
		N/A	9		

25	Has he/she ever been able to write?	No	0	Yes, name only or copies	1
		DK	8	Yes, a little	2
		N/A	9	Yes, writes well	3
26	Has he/she ever been able to add up?	No	0	Yes, a little	1
		DK	8	Yes, good at sums	2
		N/A	9		

C INDEPENDENT LIVING

27	Has he/she ever been able to choose what to wear and dress him/herself?	No	0	Yes, with support	1
		DK	8	Yes, independently	2
		N/A	9		
28	Has he/she ever been able to prepare hot drinks or basic meals?	No	0	Yes, with support	1
		DK	8	Yes, independently	2
		N/A	9		
29	Has he/she ever been able to travel on public transport?	No	0	Yes, with support	1
		DK	8	Yes, independently	2
		N/A	9		
30	Has he/she ever been able to do housework e.g. dusting, dishwashing etc.?	No	0	Yes, with support	1
		DK	8	Yes, independently	2
		N/A	9		
31	Has he/she ever been able to go shopping?	No	0	Yes, accompanied	1
		DK	8	Yes, independently for small purchases only	2
		N/A	9	Yes, independently	3
32	Has he/she ever been able to use the telephone?	No	0	Yes, answers only	1
		DK	8	Yes, dials well known numbers	2
		N/A	9	Yes, independently	3

Part 2 Cognitive and Functional Decline

Instructions to be read to informant:

"The aim of the next set of questions, is to find out about changes in’s abilities, personality and behaviour. These changes do not always appear in later life and may not be relevant to him/her, but we ask everyone the same questions because the replies might prove valuable in helping people who do have difficulties. Each question has two parts. First I will ask you whether he/she has a problem in a particular area of function; then I will ask you whether this is a deterioration or whether he/she has always had difficulty in this area."

Questions are divided into subsections according to different areas of function, that are directly linked to diagnostic criteria for dementia on page 53 of this pack.

For each question, if no difficulty is established in part a) code part b) as 9 and move on to the next question.

Where deterioration is present, please record any examples given.

A EVERYDAY SKILLS

33	<p>a) Does he/she have difficulties with his/her usual daytime activities at work, college or day centre?</p> <p style="margin-left: 20px;">Yes 1 No 0 DK 8 N/A 9</p> <p style="margin-left: 20px;"><i>Examples of change:</i></p>	→	<p>b) Is this a deterioration?</p> <p style="margin-left: 20px;">Yes → Slight deterioration 1 No 0 DK 8 N/A 9</p> <p style="margin-left: 20px;">Great deterioration 2</p>
34	<p>a) Does he/she have difficulty with a special skill or hobby?</p> <p style="margin-left: 20px;">Yes 1 No 0 DK 8 N/A 9</p> <p style="margin-left: 20px;"><i>Examples of change:</i></p>	→	<p>b) Is this a deterioration?</p> <p style="margin-left: 20px;">Yes → Slight deterioration 1 No 0 DK 8 N/A 9</p> <p style="margin-left: 20px;">Great deterioration 2</p>
35	<p>a) Does he/she have difficulty with shopping?</p> <p style="margin-left: 20px;">Yes 1 No 0 DK 8 N/A 9</p> <p style="margin-left: 20px;"><i>Examples of change:</i></p>	→	<p>b) Is this a deterioration?</p> <p style="margin-left: 20px;">Yes → Slight deterioration 1 No 0 DK 8 N/A 9</p> <p style="margin-left: 20px;">Great deterioration 2</p>

36	a) Does he/she have difficulty making a cup of tea?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No			
		DK	8			DK			
		N/A	9			N/A			
	<i>Examples of change:</i>								
37	a) Does he/she have difficulty with housework e.g. dusting, dishwashing?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No			
		DK	8			DK			
		N/A	9			N/A			
	<i>Examples of change:</i>								
38	a) Does he/she have difficulty preparing simple meals / snacks?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No			
		DK	8			DK			
		N/A	9			N/A			
	<i>Examples of change:</i>								
39	a) Does he/she have difficulty using the telephone?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No			
		DK	8			DK			
		N/A	9			N/A			
	<i>Examples of change:</i>								

If there is no deterioration in everyday skills skip to section B and code questions 40 and 41 as 9

40	How long ago did you first notice this deterioration in everyday skills? <i>Record number of months and code as applicable</i>	_____ months	< 6 Months	0	> 6 Months	1
			DK	8		
			N/A	9		
41	Did the deterioration happen gradually or did it come on suddenly?		Sudden	0	Gradual	1
			DK	8		
			N/A	9		

B MEMORY AND ORIENTATION**Memory**

42	a) Does he/she have difficulty remembering recent events e.g. when he/she last saw you or what happened the day before?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No		0	Great deterioration
		DK	8			DK			
		N/A	9			N/A			
	<i>Examples of change:</i>								
43	a) Does he/she often have difficulty remembering where he/she has left things?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No		0	Great deterioration
		DK	8			DK			
		N/A	9			N/A			
	<i>Examples of change:</i>								
44	a) Does he/she have difficulty remembering what has been said and repeat the same question over and over?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No		0	Great deterioration
		DK	8			DK			
		N/A	9			N/A			
	<i>Examples of change:</i>								
45	a) Does he/she have difficulty in remembering short lists of items, e.g. shopping?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No		0	Great deterioration
		DK	8			DK			
		N/A	9			N/A			
	<i>Examples of change:</i>								
46	a) Does he/she have difficulty remembering significant events from his/her past?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No		0	Great deterioration
		DK	8			DK			
		N/A	9			N/A			
	<i>Examples of change:</i>								

47	a) Does he/she have difficulty remembering the names of close friends, relatives or carers?	Yes	1	➔	b) Is this a deterioration?	Yes	➔	Slight deterioration	1		
		No	0			No	0			Great deterioration	2
		DK	8			DK	8				
		N/A	9			N/A	9				
		<i>Examples of change:</i>									

Orientation

48	a) Does he/she have difficulty in interpreting surroundings, e.g. knowing where he/she is?	Yes	1	➔	b) Is this a deterioration?	Yes	➔	Slight deterioration	1		
		No	0			No	0			Great deterioration	2
		DK	8			DK	8				
		N/A	9			N/A	9				
		<i>Examples of change:</i>									
49	a) Does he/she have difficulty finding the way around the neighbourhood, e.g. to the shops or Post Office near home?	Yes	1	➔	b) Is this a deterioration?	Yes	➔	Slight deterioration	1		
		No	0			No	0			Great deterioration	2
		DK	8			DK	8				
		N/A	9			N/A	9				
		<i>Examples of change:</i>									
50	a) Does he/she have difficulty finding the way around the home (or ward), e.g. finding the toilet?	Yes	1	➔	b) Is this a deterioration?	Yes	➔	Slight deterioration	1		
		No	0			No	0			Great deterioration	2
		DK	8			DK	8				
		N/A	9			N/A	9				
		<i>Examples of change:</i>									
51	a) Does he/she have difficulty knowing what day it is?	Yes	1	➔	b) Is this a deterioration?	Yes	➔	Slight deterioration	1		
		No	0			No	0			Great deterioration	2
		DK	8			DK	8				
		N/A	9			N/A	9				
		<i>Examples of change:</i>									

52	a) Does he/she have difficulty knowing what time of day it is?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1	
		No	0			No		0	Great deterioration	2
		DK	8			DK		8		
		N/A	9			N/A		9		
<i>Examples of change:</i>										

If there are no changes in memory and orientation skip to section C and code questions 53 - 56 as 9

53	How long ago did you first notice this deterioration in memory and orientation skills?	_ _ months		< 6 Months	0	> 6 Months	1
				DK	8		
				N/A	9		
<i>Record number of months and code as applicable</i>							
54	Did the deterioration happen gradually or did it come on suddenly?			Sudden	0	Gradual	1
				DK	8		
				N/A	9		
55	Do these changes interfere with his/her everyday activities?			No	0	Yes	1
				DK	8		
				N/A	9		
56	Do you think that he/she is aware of this memory problem?			No	0	Yes	1
				DK	8		
				N/A	9		

C1 OTHER COGNITIVE SKILLS

General Mental Functioning

57	a) Does he/she find it difficult to keep his/her mind on things? Is he/she easily distracted?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1	
		No	0			No		0	Great deterioration	2
		DK	8			DK		8		
		N/A	9			N/A		9		
<i>Examples of change:</i>										

58	a) Does his/her thinking seem slow?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0		
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								
59	a) Does his/her thinking seem muddled?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0		
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								

Language

60	a) Does he/she have difficulty with reading?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0		
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								
61	a) Does he/she have difficulty in following instructions?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0		
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								
62	a) Does he/she have difficulty in keeping up with ordinary conversation?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0		
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								

63	a) Does he/she have difficulty with writing?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
<i>Examples of change:</i>									
64	a) Does he/she speak very little?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
<i>Examples of change:</i>									
65	a) When speaking, does he/she have difficulty in finding the right word or use wrong words?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
<i>Examples of change:</i>									

Perception

66	a) Does he/she have difficulty identifying or recognising objects?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
<i>Examples of change:</i>									
67	a) Does he/she have difficulty identifying or recognising people?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
<i>Examples of change:</i>									

Praxis

68	a) Does he/she have difficulty carrying out familiar complex tasks (such as getting dressed) despite the physical ability to do them? <i>Examples of change:</i>	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1	
		No	0			No		0	Great deterioration	2
		DK	8			DK		8		
		N/A	9			N/A		9		

Executive Functions

69	a) Does he/she have difficulty in planning ahead and thinking about the future? <i>Examples of change:</i>	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1	
		No	0			No		0	Great deterioration	2
		DK	8			DK		8		
		N/A	9			N/A		9		
70	a) Does he/she find it difficult to make decisions? <i>Examples of change:</i>	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1	
		No	0			No		0	Great deterioration	2
		DK	8			DK		8		
		N/A	9			N/A		9		
71	a) Does he or she have difficulty solving day-to-day problems? <i>Examples of change:</i>	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1	
		No	0			No		0	Great deterioration	2
		DK	8			DK		8		
		N/A	9			N/A		9		

If there are no changes in other cognitive skills skip to section C2 and code questions 72 and 73 as 9

72	How long ago did you first notice this deterioration in abilities? <i>Record number of months and code as applicable.</i>	_____ months	< 6 Months	0	> 6 Months	1
			DK	8		
			N/A	9		
73	Did the deterioration happen gradually or did it come on suddenly?		Sudden	0	Gradual	1
			DK	8		
			N/A	9		

C2 PERSONALITY, BEHAVIOUR AND SELF CARE

Personality and Behaviour

74	a) Does he/she behave in a manner that leads to social difficulties? <i>Examples of change:</i>	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
75	a) Would you describe him/her as lacking in personality? <i>Examples of change:</i>	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
76	a) Does he/she show little emotion? Would you describe him/her as emotionally flat? <i>Examples of change:</i>	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		

77	a) Is he/she changeable in mood? i.e. Does he/she have rapid shifts between different emotions?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								
78	a) Does he/she show a lack of enthusiasm for his/her usual interests?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								
79	a) Is he/she often irritable or angry?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								
80	a) Does he/she show a lack of concern for other people?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								
81	a) Does he/she act impulsively, by doing the first thing that comes to mind?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								

82	a) Is he/she stubborn or perhaps a little awkward?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								
83	a) Does he/she get involved in difficult or embarrassing situations in public because of his/her behaviour?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								
84	a) Does he/she engage in inappropriate sexual behaviour?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								
85	a) Is he/she very restless? For example does he/she find it hard to sit down for any length of time?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								
86	a) Does he/she repeat the same word or phrase over and over again?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1
		No	0			No	0	Great deterioration	2
		DK	8			DK	8		
		N/A	9			N/A	9		
	<i>Examples of change:</i>								

87	Does he/she develop routines from which he/she cannot easily be discouraged?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1		
		No	0			No		0		Great deterioration	2
		DK	8			DK		8			
		N/A	9			N/A		9			
<i>Examples of change:</i>											
88	a) Does he/she often try to eat far too much?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1		
		No	0			No		0		Great deterioration	2
		DK	8			DK		8			
		N/A	9			N/A		9			
<i>Examples of change:</i>											
89	a) Does he/she try to eat peculiar things, such as soap, cigarettes or dirt?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1		
		No	0			No		0		Great deterioration	2
		DK	8			DK		8			
		N/A	9			N/A		9			
<i>Examples of change:</i>											

If there are no changes in personality skip to question 93 and code questions 90 – 92 as 9

90	How long ago did you first notice these changes in personality and/or behaviour? <i>Record number of months and code as applicable.</i>	_ _ months		< 6 Months	0	> 6 Months	1
				DK	8		
				N/A	9		
91	Have these changes developed gradually or did they come on suddenly?			Sudden	0	Gradual	1
				DK	8		
				N/A	9		
92	Do you think that he/she is aware of these problems?			No	0	Yes	1
				DK	8		
				N/A	9		

Self Care

93	a) Does he/she have difficulty feeding him/herself?					
	Yes, has to be fed	1	} →	b) Is this a deterioration?	Yes →	Slight deterioration 1
	Yes, eats simple solids e.g. biscuits	2			Great deterioration 2	
	Yes, eats messily with spoon only	3				
	No	0	No	0		
DK	8	DK	8			
N/A	9	N/A	9			
94	a) Does he/she have difficulty in dressing or undressing?					
	Yes, needs major assistance	1	} →	b) Is this a deterioration?	Yes →	Slight deterioration 1
	Yes, needs moderate assistance	2			Great deterioration 2	
	Yes, needs minor assistance	3				
	No	0	No	0		
DK	8	DK	8			
N/A	9	N/A	9			
95	a) Does he/she have difficulty with grooming, e.g. combing hair, shaving?					
	Yes, needs major assistance	1	} →	b) Is this a deterioration?	Yes →	Slight deterioration 1
	Yes, needs moderate assistance	2			Great deterioration 2	
	Yes, needs minor assistance	3				
	No	0	No	0		
DK	8	DK	8			
N/A	9	N/A	9			
96	a) Does he/she have difficulty with bathing or showering?					
	Yes, needs major assistance	1	} →	b) Is this a deterioration?	Yes →	Slight deterioration 1
	Yes, needs moderate assistance	2			Great deterioration 2	
	Yes, needs minor assistance	3				
	No	0	No	0		
DK	8	DK	8			
N/A	9	N/A	9			

97	a) Does he/she wet or soil him/herself?				
	Yes, is doubly incontinent	1	} →	b) Is this a deterioration?	Yes → Slight deterioration 1 Great deterioration 2
	Yes, wets often	2			
	Yes, wets occasionally	3			
	No	0	No	0	
	DK	8	DK	8	
N/A	9	N/A	9		

If there are no changes in self care skills skip to section D and code questions 98 and 99 as 9

98	How long ago did you first notice this deterioration in self care skills?	____ ____ months	< 6 Months	0	> 6 Months	1
	<i>Record number of months and code as applicable.</i>		DK	8		
			N/A	9		
99	Has this deterioration happened gradually or did it come on suddenly?		Sudden	0	Gradual	1
			DK	8		
			N/A	9		

D GENERAL SUMMARY

If no cognitive or functional decline has been established, skip to part 3 and code 100 - 102 as 9

100	Since the onset of the difficulties we have talked about, has he/she had difficulty with mobility?	No	0	Yes	1
		DK	8		
		N/A	9		
101	Can you tell me what was the first change you noticed?	DK	8	Memory	1
	<i>Record answer in full</i>	N/A	9	Other cognitive	2
				Personality	3
				Everyday Skills	4
				Other	5
102	How long ago did this first occur?	DK	8	____ ____ months	
		N/A	9		

Part 3 Current Mental Health

Instructions to be read to informant:

"The aim of the next set of questions, is to find out about 's mental health."

A DEPRESSION

103	a) Does he/she show a lack of interest or enjoyment in things in general?	Yes 1 No 0 DK 8 N/A 9	➔	b) Is this a change?	No 0 DK 8 N/A 9	Yes 1
104	a) Does he/she prefer to remain on his/her own rather than seek the company of others?	Yes 1 No 0 DK 8 N/A 9	➔	b) Is this a change?	No 0 DK 8 N/A 9	Yes 1
105	a) Is he/she lacking in energy? Does he/she find it hard to get things done?	Yes 1 No 0 DK 8 N/A 9	➔	b) Is this a change?	No 0 DK 8 N/A 9	Yes 1
106	a) Does he/she have difficulty in getting to sleep?	Yes 1 No 0 DK 8 N/A 9	➔	b) Is this a change?	No 0 DK 8 N/A 9	Yes 1
107	a) Does he/she wake early in the morning and fail to get to sleep again?	Yes 1 No 0 DK 8 N/A 9	➔	b) Is this a change?	No 0 DK 8 N/A 9	Yes 1
108	a) Does he/she sleep a lot by day?	Yes 1 No 0 DK 8 N/A 9	➔	b) Is this a change?	No 0 DK 8 N/A 9	Yes 1

109	a) Does he/she often cry?	Yes	1	→ b) Is this a change?	No	0	Yes	1
		No	0		DK	8		
		DK	8		N/A	9		
		N/A	9					
110	Does he/she talk more slowly than is usual for him/her?	No	0		No	0	Yes	1
		DK	8		DK	8		
		N/A	9		N/A	9		
111	Has he/she lost his/her appetite or become much more hungry than usual?	No	0		No	0	Yes	1
		DK	8		DK	8		
		N/A	9		N/A	9		
112	Do you think he/she is depressed?	No	0		No	0	Yes	1
		DK	8		DK	8		
		N/A	9		N/A	9		

If no depression, skip to section B and code 113 – 118 as 9

113	How long has this depression been present?	_ _ months			< 6 months	0	> 6 months	1
					DK	8		
					N/A	9		
114	Is there any reason why he/she has become depressed?				No	0	Yes, bereavement	1
					DK	8	Yes, other	2
					N/A	9		
<i>Specify:</i>								
115	Is the depression so bad that it affects every part of his/her life, friendship or family life?				No	0	Yes	1
					DK	8		
					N/A	9		
116	When he/she is feeling depressed, can anything cheer him/her up?				No	0	Yes	1
					DK	8		
					N/A	9		
117	Is depression worse in the morning?				No	0	Yes	1
					DK	8		
					N/A	9		
118	Does he/she feel other people are to blame for his/her unhappiness?				No	0	Yes	1
					DK	8		
					N/A	9		

B ANXIETY

119	a) Does he/she tend to worry a lot about little things?	Yes	1	→	b) Is this a change?	No	0	Yes	1
		No	0			DK	8		
		DK	8			N/A	9		
		N/A	9						
120	a) Have there been times lately when he/she was very anxious or frightened?	Yes	1	→	b) Is this a change?	No	0	Yes	1
		No	0			DK	8		
		DK	8			N/A	9		
		N/A	9						
<i>Describe:</i>									
121	a) Are there particular situations that make him/her anxious, e.g. going into shops or crowds?	Yes	1	→	b) Is this a change?	No	0	Yes	1
		No	0			DK	8		
		DK	8			N/A	9		
		N/A	9						
<i>Describe:</i>									
122	a) Has he/she suffered from panic attacks, when he/she felt he/she would collapse or lose control of him/herself?	Yes	1	→	b) Is this a change?	No	0	Yes	1
		No	0			DK	8		
		DK	8			N/A	9		
		N/A	9						

If no anxiety, skip to section D3 and code 124 and 125 as 9

123	How long has this anxiety been present? <i>Record number of months and code as applicable.</i>	_ _ months		< 6 Months	0	> 6 Months	1
				DK	8		
				N/A	9		
124	Have these changes developed gradually or did they come on suddenly?			Sudden	0	Gradual	1
				DK	8		
				N/A	9		

C PARANOID ILLNESS

125	a) Has he/she complained unjustifiably of being persecuted or spied upon?	Yes	1	→	b) Is this a change?	No	0	Yes	1
		No	0			DK	8		
		DK	8			N/A	9		
		N/A	9						

126	a) Has he/she been troubled by voices or visions not experienced by others?	Yes	1	→	b) Is this a deterioration?	Yes	→		
		No	0			No			0
		DK	8			DK			8
		N/A	9			N/A			9

If no paranoid illness, skip to section D and code 127 and 128 as 9

127	How long has he/she experienced this?	_ _ months		< 6 Months	0	> 6 Months	1
				DK	8		
				N/A	9		
128	Does he/she believe these are real?	No	0	Yes	1		
		DK	8				
		N/A	9				

D CLOUDING / DELIRIUM

129	a) Has there been an abrupt change towards mental confusion in recent weeks or months?	Yes	1	→	b) Is this a deterioration?	Yes	→	Slight deterioration	1	
		No	0			No		0	Great deterioration	2
		DK	8			DK		8		
		N/A	9			N/A		9		

If no confusion, skip to Part 4 on Physical Health and code questions 130 - 135 as 9

130	How long has this confusion been present?	< 6 Months	0	> 6 Months	1
		DK	8		
		N/A	9		
131	Are there periods lasting days or weeks when his/her thinking still seems quite clear?	No	0	Yes	1
		DK	8		
		N/A	9		
132	Are there brief episodes during 24 hours when he/she seems much worse and then times when quite clear?	No	0	Yes	1
		DK	8		
		N/A	9		
133	Does he/she become completely normal when the confusion clears?	No	0	Yes	1
		DK	8		
		N/A	9		
134	Is the confusion worse towards dusk or evening?	No	0	Yes	1
		DK	8		
		N/A	9		
135	Has the present illness tended recently to vary a lot, day to day, week to week, becoming worse and then improving for a while?	No	0	Yes	1
		DK	8		
		N/A	9		

E SUBSTANCE ABUSE

136	Does he/she have a significant history of alcohol or other substance abuse?	No	0	Yes	1
		DK	8		
		N/A	9		

Part 4 Current Physical Health

Instructions to be read to informant:

"The aim of the next set of questions, is to find out about’s physical health."

A PHYSICAL DISABILITY

137	Does he/she suffer from poor hearing that interferes with day to day living?	No DK N/A	0 8 9	Yes	1
138	If "yes", for how long has this been a problem?	DK N/A	88 99	_ _	months
139	a) Does he/she suffer from poor eyesight that interferes with day to day living?	No DK N/A	0 8 9	Yes	1
140	If "yes", for how long has this been a problem?	DK N/A	88 99	_ _	months
141	a) Does he/she suffer from other physical problems that interfere with day to day living? <i>Please specify:</i>	No DK N/A	0 8 9	Yes	1
142	If "yes", for how long has this been a problem?	DK N/A	88 99	_ _	months

B HYPOTHYROIDISM

143	a) Does he/she often feel the cold?	Yes	1	→	b) Is this a change?	No	0	Yes	1
		No	0			DK	8		
		DK	8			N/A	9		
		N/A	9						
144	a) Does he/she have dry skin?	Yes	1	→	b) Is this a change?	No	0	Yes	1
		No	0			DK	8		
		DK	8			N/A	9		
		N/A	9						
145	a) Does he/she have dry/brittle hair?	Yes	1	→	b) Is this a change?	No	0	Yes	1
		No	0			DK	8		
		DK	8			N/A	9		
		N/A	9						
146	a) Does he/she seem to have slowed down?	Yes	1	→	b) Is this a change?	No	0	Yes	1
		No	0			DK	8		
		DK	8			N/A	9		
		N/A	9						
147	a) Has he/she gained weight?	Yes	1	→	b) Is this a change?	No	0	Yes	1
		No	0			DK	8		
		DK	8			N/A	9		
		N/A	9						
148	Has he/she ever been told by a doctor that he/she has an under-active thyroid?					No	0	Yes	1
						DK	8		
						N/A	9		

C CEREBROVASCULAR PROBLEMS

149	Has he/she ever passed out and then had a brief weakness or difficulty with speech memory or vision?					No	0	Yes	1
						DK	8		
						N/A	9		
150	How long ago did this first occur?					DK	8	_____ months	
						N/A	9		
151	Has he/she fallen or been close to falling?					No/rarely	0	Once a month or more	1
						DK	8		
						N/A	9		
152	How long ago did this first occur?					DK	8	_____ months	
						N/A	9		

153	Has she ever had a stroke?	No	0	Yes	1
		DK	8		
		N/A	9		
154	How long ago did this first occur?	DK	8	_____ months	
		N/A	9		

D OTHER PHYSICAL ILLNESS / MEDICATION

155	Does he/she suffer from any other illness that interferes with day to day living? <i>Describe:</i>	No	0	Yes	1
		DK	8		
		N/A	9		
156	If "yes", for how long has this been a problem?	DK	88	_____ months	
		N/A	99		
157	Does he/she currently take any medication? <i>Specify:</i>	No	0	Yes	1
		DK	8		
		N/A	9		

S Hemisphere comparisons for cortical thickness data

	Brain hemisphere mean cortical thickness			Correlation between hemisphere averages	
	Right	Left	<i>p</i>	<i>r</i>	<i>p</i>
Frontal	2.548	2.615	.001	.896	.001
Parietal	2.511	2.504	.001	.902	.001
Temporal	2.958	2.914	.010	.876	.001
Occipital	2.386	2.313	.730	.848	.001

Table S.1 Paired comparison data on cortical thickness measures between hemispheres.