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The Visual Impairment/Cognitive Impairment Co-morbidity: Examining the Genotype-Structure-Function Relationship

by

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Résumé

Un nombre de recherches rapportent une forte cooccurrence de la dégénérescence maculaire liée à l'âge (DMLA) et la maladie d'Alzheimer (AD), ce qui suggère que les déficiences visuelles et cognitives peuvent être liées. Ceci est davantage soutenu par des similitudes structurelles dans la rétine et le cerveau qui sont des facteurs de risque de maladie partagés et des preuves histopathologiques, y compris le bêta-amyloïde. En raison de cela, l'hypothèse selon laquelle DMLA et AD peuvent également partager des facteurs de risque génétiques. L'objectif de cette recherche était de reproduire des études démontrant une incidence plus élevée d'altération cognitive chez les personnes atteintes de DMLA et d'explorer la relation entre le génotype, la structure, et la fonction dans cette comorbidité.

Les résultats ont montré qu'un plus grand nombre de personnes atteintes de DMLA ont obtenu un résultat positif pour déficience cognitive par rapport aux témoins. Le résultat MoCA moyen pour le groupe DMLA était inférieur à celui du groupe témoin, mais ce n'était pas significatif. Ces résultats positifs pour déficience cognitive dans la DMLA et les groupes témoins diffèrent considérablement sur les domaines cognitifs avec lesquels ils avaient des difficultés. Bien que les contrôles aient des difficultés avec la mémoire seulement, ceux avec DMLA ont eu de la difficulté avec la mémoire en plus d'autres domaines cognitifs, ce qui indique un risque plus élevé de progression vers AD.

Les résultats génétiques ont montré que les polymorphismes de nucléotide unique (SNP), CFHY402H et ARMS2A69S de DMLA les plus fréquents se produisent dans les fréquences attendues au sein de la population québécoise. FADS1 rs174547, qui a une contribution moins significative à AMD, a été constaté surreprésenter dans la population québécoise, ce qui indique un effet fondateur pour ce SNP. En terme de fonction visuelle, les transporteurs de CFHY402H se sont révélés avoir une mauvaise stabilité de la fixation par rapport aux non-porteurs, tandis que les porteurs d'ARMSA69S avaient une acuité visuelle et une sensibilité au contraste plus médiocres. L'analyse de la structure rétinienne a révélé que CFHY402H était liée à l'augmentation de la zone de Drusen, à la réflexivité moyenne et à l'atrophie géographique, tandis que l'ARMS2A69S

avait moins de corrélations avec les caractéristiques du Drusen. Ensemble, ces résultats suggèrent que le SNP de CFH joue un rôle dans la perturbation de l'architecture de la rétine alors que le SNP ARMS2 est impliqué dans le dysfonctionnement des photorécepteurs. Ceci est encore mis en évidence par les résultats des mesures psychophysiques, où les porteurs d'ARMS2A69S avaient une difficulté particulière avec les stimuli de premier ordre qui dépendent fortement de la sensibilité au contraste. Bien qu'aucune différence significative n'a été trouvée dans la performance cognitive basée sur le statut de transporteur CFH ou ARMS2, tous ceux qui ont obtenu une évaluation positive pour une déficience cognitive étaient des porteurs du SNP FADS1 avec des homozygotes ayant les scores cognitifs les plus bas.

Ces résultats ont des répercussions sur les domaines de la génétique, de la biologie et de la rééducation à faible vision. En explorant la comorbidité cognitive de DMLA dans l'ensemble du spectre de la fonction génotype-structure, la communication à travers les sciences augmente pour mieux servir la population croissante confrontée à cette comorbidité.

Mots clés: La dégénérescence maculaire liée à l'âge (DMLA), la maladie d'Alzheimer (AD), la déficience cognitive légère, le facteur de complément H (CFH), la susceptibilité à la maculopathie liée à l'âge 2 (ARMS2), la désaturase acide gras 1 (FADS1), le nucléotide unique polymorphisme (SNP), drusen, retina, fonction visuelle

Abstract

Research reports a high co-occurrence of Age-related Macular Degeneration (AMD) and Alzheimer's Disease (AD), suggesting that visual and cognitive impairments may be related. This is further supported by structural similarities in the retina and brain, shared disease risk factors, and histopathological evidence, including beta-amyloid. Due to this, it is hypothesized that AMD and AD may share genetic risk factors as well. The goal of this research was to replicate studies demonstrating a higher incidence of cognitive impairment among individuals with AMD, and to explore the relationship among genotype, structure, and function in this co-morbidity.

The results showed a greater number of individuals with AMD scored positive for mild cognitive impairment (MCI) compared to controls. Mean Montreal Cognitive Assessment score for the AMD group was lower than that of the control group, however this was not significant. Those scoring positive for MCI in the AMD and control groups did differ significantly on the cognitive domains with which they had difficulty. While controls had difficulty with only memory, those with AMD had difficulty with memory in addition to other cognitive domains, indicating a higher risk of progression to AD.

The genetic results showed that the most common AMD single nucleotide polymorphisms (SNPs), CFHY402H and ARMS2A69S, occur in the expected frequencies within the Quebec population. FADS1 rs174547, which has a less significant contribution to AMD, was found to be overrepresented in the Quebec population, indicating a possible Founder Effect for this SNP. In terms of visual function, carriers of CFHY402H were found to have greater ecentricity compared to non-carriers while carriers of ARMSA69S had poorer visual acuity and contrast sensitivity. Analysis of retinal structure revealed CFHY402H was related to increased drusen area, mid reflectivity, and geographic atrophy, meanwhile ARMS2A69S had fewer correlations with characteristics of drusen. Taken together, these results suggest that the CFH SNP plays a role in the disruption of retinal architecture while the ARMS2 SNP is involved in photoreceptor dysfunction. This is further evidenced by the results of psychophysical measures, where carriers of ARMS2A69S had particular difficulty with first order stimuli

which relies heavily on contrast sensitivity. Although no significant differences were found in cognitive performance based on CFH or ARMS2 carrier status, all those scoring positive for MCI were carriers of the FADS1 SNP with homozygotes having the lowest cognitive scores.

These results have implications for the fields of genetics, biology, and low vision rehabilitation. Exploration of the AMD/cognitive impairment co-morbidity across the spectrum of genotype-structure-function increases communication across the sciences to better serve the growing proportion of the population facing this co-morbidity.

Keywords: Age-related Macular Degeneration (AMD), Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), Complement Factor H (CFH), Age-related Maculopathy Susceptibility Gene 2 (ARMS2), Fatty Acid Desaturase 1 (FADS1), Single nucleotide polymorphism (SNP), Drusen, Retina, Visual function

Table of Contents

List of Tables

List of Figures

List of Abbreviations

3DMOT three-dimensional multiple object tracking

βA beta-amyloid

AA arachidonic acid

ACh acetylcholine

AChE acetylcholinesterase

AD Alzheimer's Disease

ALA alpha-linoleic acid

AMD Age-related Macular Degeneration

ApoE apolipoprotein E

APP apolipoprotein precursor protein

AREDS Age-related Eye Disease Study

ARMS2 Age-related Macular Susceptibility Gene 2

ANCOVA analysis of covariance

ANOVA analysis of variance

BCEA bivariate contour ellipse area

BMI body mass index

CFH Complement Factor H

CNS central nervous system

CNV choroidal neovascularization

CRP C-Reactive Protein

CS contrast sensitivity

dbSNP Single Nucleotide Polymorphism Database

DHA docosahexaenoic acid

EPA eicosapentaenoic acid

ETDRS Early Treatment of Diabetic Retinopathy Study

FA fluorescein angiography

FADS1 Fatty Acid Desaturase 1

fMRI functional magnetic resonance imaging

FO first order

GWAS genome-wide association study

HDL High Density lipoprotein

MCI mild cognitive impairment

MoCA Montreal Cognitive Assessment

MORE-LVR Memory or Reasoning Enhanced Low Vision Rehabilitation

MMSE Mini Mental-State Exam

MWU Mann-Whitney U test

nAChR nicotinic acetylcholine receptor

OCT optical coherence tomography

OCT/SLO optical coherence tomographer/scanning laser ophthalmoscope

PRL preferred retinal locus

PUFA polyunsaturated fatty acid

RGC retinal ganglion cell

RNFL retinal nerve fiber layer

ROS reactive oxygen species

RPD reticular pseudodrusen

SLO scanning laser ophthalmoscope

SNP single nucleotide polymorphism

SO second order

VA visual acuity

VEGF vascular endothelial growth factor

For my Gran, Margaret MacKay

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Chapter 1: Introduction and Literature Review

Background

As the Canadian population ages, the shift in demographics is reflected by an increase in age-related health problems such as Age-related Macular Degeneration (AMD) and Alzheimer's Disease (AD). According to the National Eye Institute, AMD affects 2.5% of the population over 50, with a prevalence that increases with age and with up to 35% of the population above 80 being diagnosed with intermediate or advanced stages of the disease. It is presently the leading cause of severe visual impairment in industrialized nations (Klein et al., 2007). According to the World Health Organization, dementia affects 5-8% of people over the age of 60. AD is currently the leading cause of dementia, accounting for approximately 60-70% of cases (Burns & Iliffe, 2009). While AMD affects abilities such as reading and driving, AD reduces one's ability to understand and to communicate. Thus, both conditions severely affect quality of life, independence and social participation. Currently, the causes of these ailments are not well understood and there are few, if any, effective treatments. As life expectancy increases and the population ages, more cases will develop.

Age-related Macular Degeneration (AMD)

AMD is a degenerative condition with few available treatment options. The disease is classified into "Early" and "Late" stages. Early AMD involves the development of soft drusen - yellowish deposits between the RPE and Bruch's membrane that are composed of protein and lipid debris. Small, hard drusen that develop in the periphery are typical with age, while soft drusen in the area of the macula indicate the development of AMD (Ding, Patel, & Chan, 2009; Katta, Kaur, & Chakrabarti, 2009). Soft drusen occur in large numbers, tend to be larger in size and have irregular borders. Patients often perceive them as fuzzy cotton-like spots in their visual field. The early stage of AMD is also accompanied by the thickening and loss of normal architecture in Bruch's membrane (Ding et al., 2009; Pappuru et al., 2011). At this stage patients typically experience some

loss of central vision and have trouble with contrast sensitivity and spatiotemporal sensitivity (Katta et al., 2009).

Late stage AMD consists of two phenotypes: the atrophic (dry) form and the exudative (wet form). Dry AMD is the most common, accounting for 85-90% of cases. At this stage, soft drusen become so large and numerous that they begin to coalesce preventing the transport of nutrients supplied by the choroidal capillaries. The lack of nutrition causes the death of photoreceptor cells and a thinning of the RPE layer, referred to as geographic atrophy (Katta et al., 2009). The slow degeneration of the RPE occurs over a number of years gradually blurring central vision (Campochiaro, 2011). Presently, there is no treatment for the dry form of AMD. Removal of drusen by laser has been attempted (Group, 2003; Owens, Bunce, Brannon, Wormald, & Bird, 2003; Owens, Guymer, Gross-Jendroska, & Bird, 1999), but the results were not conclusively prophylactic or restorative (Owens et al., 2003). Initially, the intake of antioxidant vitamin and mineral supplements as indicated by the Age-Related Eye Disease Study (AREDS) study was shown to decrease the risk of progression to late stage AMD, but research as of late has been controversial (Rojas-Fernandez & Tyber, 2016; Seddon, Silver, & Rosner, 2016).

Wet AMD comprises 10% of cases. It is considered the more severe form due to the rapid loss of vision associated with it. It is characterized by choroidal neovascularization (CNV), the growth of new and fragile vasculature. These vessels break through Bruch's membrane into the sub-retinal pigment epithelium and are prone to leaking or haemorrhaging due to their lack of tight junctions. Blood and/or fluid in this region of the eye can lead to retinal detachment or destruction of photoreceptors. Fluid-filled regions block the delivery of oxygen and nutrients to the RPE resulting in massive cell death and the development of dense scars in the macula (Campochiaro, 2011; Ding et al., 2009; Francis & Klein, 2011; Katta et al., 2009; Wells et al., 1996). The best current treatment for wet AMD consists of intraocular injections of anti-Vascular Endothelial Growth Factor (anti-VEGF) to prevent the growth of new vasculature.

Alzheimer's Disease (AD)

AD, also a progressive, degenerative disorder, is characterized by a gradual decline in cognitive function. Early stages are marked by short-term memory loss that can evolve into language problems, disorientation, moodiness, loss of ability to care for oneself and behavioural issues such as aggression (Burns & Iliffe, 2009). Due to the nature and slow progression of the disease, it is likely that neurodegeneration begins long before the clinical manifestation of symptoms. This pre-clinical phase is recognized as a separate entity: Mild Cognitive Impairment (MCI). Individuals identified as having MCI are at a higher risk of progressing to AD, but this prognosis is not absolute.

Anatomically, AD brains show shrinkage of the cerebral cortex and hippocampus and enlargement of the ventricles. These changes are accompanied by histopathological abnormalities including neuronal loss, neurofibrillary tangles (Braak & Braak, 1996; Grundke-Iqbal, Iqbal, Quinlan, et al., 1986; Grundke-Iqbal, Iqbal, Tung, et al., 1986), granulovascular degeneration and senile plaques containing β-amyloid (βA) (Hardy & Selkoe, 2002). These changes typically affect the hippocampus and limbic structures leaving the motor and visual cortices relatively spared (Hinton, Sadun, Blanks, & Miller, 1986, 2010). Patients can be tentatively diagnosed using a combination of neuropsychological tests and brain imaging techniques. Studies investigating the identification of cerebrospinal biomarkers for AD are underway (Sui, Liu, & Yang, 2014), but, currently, a definitive diagnosis is only achieved through post-mortem study of the brain. Due to the slow progression of the disease and known treatment being most effective at early stages, research efforts are focused on the search for biomarkers in order to detect AD and monitor progression/treatment (Cummings, 2011).

The major sites of AMD pathology are the retinal neurons, especially photoreceptors, implying that AMD, much like AD, may be a neurodegenerative disorder associated with aging. Research has established that AMD and AD are complex multifactorial diseases influenced by genetics, lifestyle choices and the individual's environment. Recent studies have found a higher than expected co-occurrence of these ailments leading to investigations that identified common histopathology and raised

questions about similar pathologic mechanisms and underlying genetic factors. With the sequencing of the human genome, the development of non-invasive imaging techniques and sophisticated psychophysical tools, scientists can study the aetiology of complex human diseases like AMD and AD from the molecular level all the way to functional manifestation. This dissertation aims to explore the visual impairment/cognitive impairment co-morbidity in terms of genotype, retinal structure and visual/cognitive function.

Literature Review

The retina and brain have a lot in common. Philosophers and poets were saying that "the eye is the window to the soul" long before researchers explored the scientific basis of the metaphor. Anatomically, the retina is a sensory extension of the central nervous system (CNS). In embryology, the retina and the optic nerve extend from the diencephalon and are considered part of the brain (Chang et al., 2014; London, Benhar, & Schwartz, 2012). Axons from the Retinal Nerve Fiber Layer (RNFL) and optic nerve synapse directly with several brain regions. The primary units of the retina and brain, the retinal ganglion cells (RGCs) and CNS neurons respectively, share structural properties as well. Both cell types are composed of a central cell body, dendrites, which receive signals, and axons, which propagate the signal. Posterior to the globe, RGC axons are covered by a myelin sheath much like CNS neurons (London et al., 2012). RGCs behave like CNS neurons when suffering from insult. There is anterograde and retrograde degeneration of severed axons, myelin destruction, scar formation and creation of a neurotoxic environment involving oxidative stress, abnormal aggregation of debris, toxic levels of neurotransmitters and deprivation of neurotrophic factors. Much of what we have learned about CNS nerve regeneration has come from studying the optic nerve (London et al., 2012).

Like the CNS, the retina is an immune-privileged site having a specialized immune response. The anterior chamber of the eye is filled with aqueous humor, a fluid that carries anti-inflammatory and immunoregulatory mediators similar to the cerebral spinal fluid that circulates around the brain and spinal cord. Blood barriers and strict

gating systems aid in the protection of these immune-privileged areas. Barriers are composed of non-fenestrated endothelial cells connected by tight junctions and reinforced by astrocyte and Müller cell processes. Pericytes, cells that wrap around endothelial cells on the inside of capillaries, play an important role in the functionality of the barrier. Through paracrine signalling and direct cell contact, they regulate capillary blood flow and vesicle trafficking and communicate with endothelial cells to regulate clearance, flow and permeability. This restricts the movement of materials from the blood to the retina and brain compared to other tissues (London et al., 2012).

Visual and Ocular Manifestations of Alzheimer's Disease

Given the similarities between the structure and properties of the brain and retina, scientists began to investigate what similarities they might have in disease states. It was found that several neurodegenerative disorders including stroke, Multiple Sclerosis, Parkinson's Disease and AD, all of which affect the brain, also have ocular manifestations (Chang et al., 2014; London et al., 2012). In terms of AD, this makes sense since visual disturbances are common complaints of patients with a probable AD diagnosis (Berisha, Feke, Trempe, McMeel, & Schepens, 2007; Kesler, Vakhapova, Korczyn, Naftaliev, & Neudorfer, 2011). These complaints include reduction in visual field, deficits in colour vision and contrast sensitivity as well as difficulty with fixation stability, motion perception, visual attention and visual memory (Ikram, Cheung, Wong, & Chen, 2012). Behavioural studies using transgenic AD mice have also confirmed visual dysfunction (Chiu et al., 2012; Parnell, Guo, Abdi, & Cordeiro, 2012). This led to the study of the ocular manifestations specific to AD.

Researchers studying changes in the vision of AD patients have found a number of visual deficits. It has been found that advanced AD, but not early stages of the disease, is associated with a loss in visual acuity (Uhlmann, Larson, Koepsell, Rees, & Duckert, 1991). It has also been reported that AD is associated with deficits in colour vision (Pache et al., 2003) and contrast sensitivity (Neargarder, Stone, Cronin-Golomb, & Oross, 2003). Furthermore, several studies indicate that there is damage to the magnocellular pathway in AD (Gilmore, Wenk, Naylor, & Koss, 1994; Hof & Morrison, 1990; Rizzo &

Nawrot, 1998) despite it being relatively clear of plaques. This is evidenced by a deficit in motion perception. After evaluation with a correlated motion paradigm, AD subjects had significantly higher motion detection thresholds compared to age-matched controls. In addition, motion thresholds correlated with disease severity with higher thresholds in more advanced cases (Gilmore et al., 1994).

There has been speculation that cortical dysfunction alone is not enough to explain the visual disturbances so often reported in AD (Coppola et al., 2015). Some studies went as far as to claim that the ocular manifestations might precede clinical disease symptoms (Koronyo-Hamaoui et al., 2011; Koronyo, Salumbides, Black, & Koronyo-Hamaoui, 2012; London et al., 2012). Visuospatial disorientation is associated with posterior cortical atrophy and impaired visual motion processing in AD. In response to neurological testing, one study demonstrated that ½ of AD patients, 1/3 of MCI patients and 1/5 of age-matched controls had impaired visual motion processing. This poor performance was not associated with verbal or memory deficits, leading the investigators to conclude that the observed visuospatial impairment may be an independent sign of neurodegenerative disease, possibly preceding clinical onset (Mapstone, Steffenella, & Duffy, 2003). This claim is supported by histopathological studies in animals. In a mouse model of AD, the plaque-labelling fluorochrome, curcumin, was used to show that retinal plaques preceded the deposition of plaques in the brain (Koronyo et al., 2012). One group proposed visual impairments may be due to plaques in the visual association cortex rather than changes to the retina or optic nerve (Rizzo & Nawrot, 1998). When the plaque density in the primary visual cortex was measured, it was found to correspond to the losses in visual field (Armstrong & Kergoat, 2015).

Retinal Manifestations

Several groups have found significant differences between the eyes of individuals with AD and those without. Hinton and colleagues were the first to report structural changes in the optic nerve head. Post-mortem analysis of AD eyes showed thinning of the optic nerve head and RGC loss resulting in optic disk cupping and pallor (Hinton et al., 1986). A healthy optic nerve head has approximately 1.2 million nerve fibers passing

through it, transmitting information between the retina and brain. The cup-to-disk ratio is a measure of empty space in the optic nerve head. A ratio of 0.5 is normal and means that there is an equivalent amount of nerve fiber tissue and empty space in the optic nerve head. Optic disk cupping occurs when the cup-to-disk ratio is higher. This indicates pathology since there is more empty space than nervous tissue (Hinton et al., 1986, 2010).

Early post-mortem findings spurred further study by other groups, this time *in vivo*, using fundus photography, optical coherence tomography (OCT), scanning laser ophthalmoscopy (SLO) or combined OCT/SLO. A 2001 study using Heidelberg retinal tomography disagreed with post-mortem findings. They were unable to detect differences in optic nerve head structure between controls and individuals with early Alzheimer's type dementia (Kergoat, Kergoat, Justino, Robillard, et al., 2001). However, another group using the SLO and fundus photography, revealed that those with AD have fewer ganglion cell axons and are more likely to have a larger cup to disc ratio compared to healthy peers (Danesh-Meyer, Birch, Ku, Carroll, & Gamble, 2006). A later study by Hinton's group examining live optic nerves showed that 8/10 AD patients had optic disk cupping. Patients had a two- to three- fold depletion of axons compared to age-matched controls. The remaining axons were not only smaller in diameter, but interspersed with increased amounts of glia compared to what were normally thicker axons arranged in bundles with infrequent glia. The replacement of dead axons with glial tissue and accompanying reduction in capillaries causes the optic nerve head to appear whiter than normal upon examination of the fundus. This is referred to as optic disk pallor. Many older adults have ophthalmic disease but even the most common optic neuropathies occur in less that 5% of the elderly population. Hinton et al. speculated that it was unlikely that 8/10 patients had bilateral optic neuropathies unrelated to AD (Hinton et al., 2010).

The reduced number of RGC axons led to imaging of the RNFL via OCT. RNFL thickness was reduced in AD and MCI patients compared to age-matched controls (Iseri, Altinas, Tokay, & Yuksel, 2006; Marziani et al., 2013; Paquet et al., 2007), particularly in the superior RNFL (Parnell et al., 2012). This was consistent with the compromised function measured in inferior visual field of AD patients during ophthalmic examination (Trick, Trick, Morris, & Wolf, 1995). Further study of the RFNL in AD patients showed

that thickness correlated with severity of AD as determined by the Mini Mental State Exam (MMSE) (Guo, Duggan, & Cordeiro, 2010).

Overall, macular volume was also affected, with AD patients demonstrating thinning in the inferior, nasal and temporal quadrants, but not the superior quadrant (Iseri et al., 2006). This was contrary to RNFL studies indicating other retinal layers may be affected. Chorioretinal thickness, measured via spectral-domain OCT, showed that macular ganglion cell complex thickness was significantly reduced in AD patients compared to controls, especially in the superior and inferior areas, however, no difference in outer retinal thickness was found. When the same group measured choroidal thickness alone, other than a region 3.0 mm temporal of fovea, thickness was severely decreased in the AD group. When Mini-Mental State Exam (MMSE) scores were compared with OCT findings, cognitive scores correlated with macular ganglion cell complex thickness, but not choroidal thickness (Bayhan, Aslan Bayhan, Celikbilek, Tanık, & Gürdal, 2015). Ascaso et al. (2014) did a similar study comparing OCT findings among AD patients, amnestic MCI (aMCI) patients and age-matched controls. aMCI is the most common early cognitive impairment that converts to AD. They too, found decreased RNFL thickness in AD patients compared to controls. An unexpected finding, however, was the aMCI group actually had the greatest macular volume of the groups followed by the controls and finally, the AD group. Researchers speculate these results could be due to inflammation and gliosis known to occur early in AD. This would explain the increase in macular volume during aMCI followed by the decrease post-AD diagnosis. Multiple regression analysis found a strong association between MMSE score and RNFL thickness, but no association between MMSE score and macular volume or thickness (Ascaso et al., 2014).

Of course, there have also been studies disputing these findings. One of these measured RNFL thickness near the optic nerve head centre in mild-moderate AD subjects and controls. No difference in RNFL thickness or distribution of optic nerve fibers was found between groups (Kergoat, Kergoat, Justino, Chertkow, et al., 2001; Kergoat, Kergoat, Justino, Robillard, et al., 2001). These conflicting results could be due to differences in methodology. No significant differences were found between AD groups

and controls in studies that used scanning laser tomography and scanning laser polarimetry rather than OCT. This is particularly true for earlier studies using earlier versions of the equipment (Chang et al., 2014). Meta-analysis of 17 studies comparing AD patients and healthy age-matched controls noted a significant reduction in mean RNFL thickness in the AD group. Of those studies, five included a MCI group. They also found a significant difference between RNFL thickness in MCI versus controls. Finally, the meta-analysis concluded that RNFL thickness as measured by OCT is diagnostically useful in discriminating between healthy, MCI and Alzheimer's states (Thomson, Yeo, Waddell, Cameron, & Pal, 2015).

Non-retinal Ocular Manifestations

The retina is not the only component of the eye that is affected in AD. A large number of AD patients have been shown to have equatorial supranuclear cataracts (Goldstein et al., 2003). Insoluble protein aggregates are a common feature of the senile plaques in AD and cataracts. This type of cataract, unlike the more common age-related type, is obscured from view by the iris. It does not disrupt the visual axis and is usually not identified during routine eye exams. Equatorial supranuclear cataracts can be viewed by pupil dilation. More research is required but, if this type of cataract were found to precede neurological symptoms in AD, screening for it may be useful in the early detection of AD (Chang et al., 2014; Goldstein et al., 2003; Liang, 2000).

AD is also characterized by oculomotor deficits. Research has found that AD is associated with increased saccade latencies (Fletcher & Sharpe, 1986; Scinto et al., 1994). Furthermore, AD patients display less efficient eye movement patterns compared to age-matched controls. They have difficulty maintaining fixation on still and moving targets (Fletcher & Sharpe, 1986). Smooth pursuit is also a problem (Pelak, 2010): AD patients tend to move their eyes in a jerky manner due to reduced accuracy followed by "catch-up" saccades. Research has also found that advanced stages of AD are associated with reductions in vergence (Uomori et al., 1993).

It is well known that there is a reduction in cerebral blood flow in AD patients. The Rotterdam Study has shown that cerebral hypoperfusion precedes the onset of

clinical dementia (Ruitenberg et al., 2005). Animal studies have speculated that this is due to senile plaques. Their accumulation causes cerebral arteries to constrict in order to make room for them (Iadecola, 2004). Whether abnormal flow leads to neuronal cell death or is a result of it is still under debate (de la Torre, 2000). More recently, Doppler Imaging was used to show that the retinal vasculature mirrors the change in blood flow in the brain (Berisha et al., 2007; Chang et al., 2014; Parnell et al., 2012). This was evident when fundus photography demonstrated that AD patients had significantly narrower vasculature compared to controls (Berisha et al., 2007).

The Co-occurrence of Visual and Cognitive Impairments

Visual and cognitive impairments are known to increase with age. Visual disturbances are among the earliest complaints of individuals with a probable AD diagnosis (Berisha et al., 2007). Studies have also shown vision impairment to be a risk factor for cognitive decline (Lin et al., 2004; Reyes-Ortiz et al., 2005). Recently, there has been a growing body of literature suggesting there may be more than just age linking the two conditions. Several studies in our lab have shown that 25-50% of study participants with AMD score positive for MCI according to the Montreal Cognitive Assessment (MoCA) (Boxerman, Wittich, & Overbury, 2015; Duponsel, Wittich, Dubuc, & Overbury, 2010) .

One early study (Wong et al., 2002) used retinal photography and the Wisconsin AMD Grading System (Klein et al., 1991) to categorize patients as having early AMD or late AMD. Cognitive function was assessed using three neuropsychological tests; the Delayed Word Recall Test, the Digit-Symbol Subtest of the Wechsler Adult Intelligence Scale Revised and the Word Fluency Test from the Multilingual Aphasia Examination. After controlling for factors such as age, gender, race, level of education and cardiovascular risk, results showed that participants with severe impairments according to the Word Fluency test were 60% more likely to have early AMD and associated lesions than those without cognitive impairment. Severe impairment on the other two neuropsychological tests was not associated with AMD grades (Wong et al., 2002).

A series of studies by Whitson *et al.* investigated the relationship between macular disease and cognitive impairment in a low vision rehabilitation setting (Whitson et al., 2007, 2010, 2011, 2013). Early investigations showed that participants with coexisting visual and cognitive impairments were at higher risk of disability according to the Instrumental Activities of Daily Living scale, with each condition contributing additively to disability risk (Whitson et al., 2007). Comparison of participant results with age-matched normative data on the Telephone Interview for Cognitive Status (modified) found that 18.8% of their participants scored positive for cognitive impairment and another 27.7% had a score near the cut-off value (Whitson et al., 2010). Further study led to the development of the Memory or Reasoning Enhanced Low Vision Rehabilitation (MORE-LVR) intervention plan to help patients with macular disease and cognitive impairment. The 6-week intervention plan consisted of three components, each specifically tailored to the recipient. The first component consisted of frequent training sessions with an occupational therapist having low vision experience. Training sessions were focused on three functional goals (reading, cooking, shopping, etc.) decided upon by the therapist and client ahead of time. The second component was a simplified training environment, meaning sessions occurred in a quiet, minimally distracting place and maintained a focused educational agenda. The final component was the involvement of an informal companion having no cognitive or visual impairments, typically a friend or family member. The purpose of the companion was to provide social interaction and to aid in the practice of what was learned during training sessions. The MORE-LVR pilot study revealed improvements in vision-related function and cognitive measures in addition to high patient satisfaction (Whitson et al., 2013). This series of studies is limited by the choice of participant population. Being from a low vision rehabilitation clinic, the focus was not strictly on AMD as participants with other macular pathologies were included. Further, those with AMD receiving rehabilitation are likely in later stages of the disease and have already had extensive vision loss. Finally, no examination of the retina was performed, preventing any investigation into the relationship between cognitive function and retinal health beyond the initial AMD diagnosis.

Several studies have investigated the relationship between AMD and cognitive impairment as determined by the MMSE (Baker et al., 2009; Harrabi et al., 2015; Pham, Kifley, Mitchell, & Wang, 2006). Pham *et al*. (2006) tested cognitive status across three different participant groups (early AMD, late AMD and non-AMD) based on evaluation of fundus photographs using the Wisconsin AMD Grading System (Klein et al., 1991). The results reveal a statistically significant relationship between cognitive impairment and late AMD (Pham et al., 2006). The significance remained even after excluding vision-related components of the MMSE. This result is supported by more recent work, which found those with retinal disease had lower MMSE scores compared to healthy controls (Harrabi et al., 2015). Pham et al., (2006) reported cognitive scoring in early AMD was not statistically different from that of the non-AMD group (Pham et al., 2006). This finding is also supported by later studies (Baker et al., 2009) that failed to find significant associations between cognitive impairment and early AMD. However, given that the MMSE is not sensitive to MCI (Dong et al., 2012; Hoops et al., 2009), it is possible that early AMD may be associated with cognitive deficits too mild to be detected by the test. Research continues to demonstrate that the MoCA is better suited to detecting MCI (Dag, Örnek, Örnek, & Türkel, 2014; Nasreddine et al., 2005).

More recent studies have investigated the relationships between AMD characteristics and cognitive impairment. Using fundus photography and the International Classification System for AMD (Bird et al., 1995), Lindekleiv et al. (2013) assigned participants to one of four groups based on AMD phenotype. The phenotype was defined based on the most severe lesion located in the macula of the worse eye: normal (drusen < 63 μm), intermediate drusen (drusen 63-125 μm), large drusen (drusen > 125 μm), and late stage AMD (presence of choroidal neovascularization or geographic atrophy). They used three types of cognitive tests to evaluate cognitive function; a digit-symbol coding task, verbal memory test and a finger-tapping task. When comparing their results to normative data, it was shown that late-stage AMD was associated with low verbal memory scores and intermediate and large drusen were associated with poor performance in the digit-symbol coding task (Lindekleiv et al., 2013).

Woo and colleagues (2012) also divided participants into groups based on a different set of characteristics: early stage and late stage. Late stage was subcategorized into neovascular (wet), geographic atrophy and polypoidal choroidal vasculopathy. The Alzheimer's Disease Neuropsychological Assessment Battery, Benton Visual Retention Test and Digit Span Test were used to evaluate cognitive function. They found that cognitive function is impaired in AMD patients and that they have a 3-fold higher risk of developing MCI compared to age-matched controls. Patients who had geographic atrophy had the worst cognitive scores (Woo et al., 2012).

Other research groups have studied the association of AMD with different cognitive domains. Those with wet AMD were found to have poorer memory performance compared to controls while those with dry AMD had poorer executive function in addition to deficits in memory (Rozzini et al., 2014). Previous research has shown that poor episodic memory paired with a decline in executive function is seen most commonly in MCI patients that progress to AD (Rozzini et al., 2007). This suggests that individuals with dry AMD may be at a higher risk of developing AD. This hypothesis was supported by a study from a Turkish population that showed a higher prevalence of AD among those with the dry form compared to wet AMD or controls (Demirci et al., 2015).

Large-scale studies investigating the occurrence of co-morbid AMD and cognitive impairment have also been conducted. An Italian study sought to reliably establish whether or not there was a relationship between cognitive impairment and age-related vision disorders. They showed a closer association of AMD with AD than with other types of dementia such as mixed or vascular. The same was true for the association of AD with other types of vision impairment (Mandas et al., 2014). An earlier and more specific endeavour, The Rotterdam Study, a prospective population-based investigation in the Netherlands, found an increased prevalence of the development of cognitive impairment in AMD subjects over a four-year period (Klaver et al., 1999). The AREDS Report No. 16 performed a cross-sectional analysis of data produced by nearly 3,000 people. They showed macular abnormalities and lower visual acuity were associated with poorer cognitive function (AREDS Research Group, 2006). This study was limited by using

strictly psychological tests and not evaluating cognitive function in detail. Late stage AMD was also considered one category instead of examining wet versus dry. The Blue Mountain Eye Study, conducted in Australia, also found a significant association between late AMD and cognitive impairment (Pham et al., 2006). They relied solely on the MMSE for cognitive testing and also did not separate the types of late-stage AMD. The Cardiovascular Health Study used the Digit Symbol Substitution Test and the Modified Mini Mental State Examination to determine cognitive status along with the modified Wisconsin AMD Grading Scheme to evaluate fundus photographs. They found a strong association between low scores on the Digit Symbol Substitution test and early AMD, but no association with AMD status and the modified Mini Mental State Exam score (Baker et al., 2009). Another population-based study, this time from Taiwan, agreed with the former two studies, showing that individuals with AMD were at a higher risk of developing AD or senile dementia, especially those with advanced dry AMD (Tsai, Chen, Huang, Yuan, & Leu, 2015).

Conversely, a large-scale study from England found that the development of cognitive impairment among those with AMD was no more prevalent than that of chance in a normal population (Keenan, Goldacre, & Goldacre, 2014). Although this study utilized a large sample population (N>200,000), patients receiving anti-VEGF treatment were recruited from hospital-based clinics. Since only the wet form of AMD receives this type of treatment, their sample lacked the dry form. Given that studies mentioned previously demonstrated a stronger link between dry AMD and cognitive impairment (Demirci et al., 2015; Rozzini et al., 2014; Tsai et al., 2015; Woo et al., 2012), recruitment from a hospital-based clinic may have introduced a sample bias.

Shared Risk Factors

AMD and AD are both complex diseases whose development is influenced by a variety of factors. The most well-established risk factor for both is age. Epidemiological studies have shown the risk of developing AMD increases three fold for those between 60 and 80 years of age compared to those under 60 (Friedman, Katz, Bressler, Rahmani, & Tielsch, 1999), while the risk for developing AD doubles every five years after the age of

65 (Qiu, Kivipelto, & Von Strauss, 2009a). During the natural aging processes several pathological changes occur that affect the integrity of retinal and neural tissues contributing to AMD and AD respectively (Katta et al., 2009; Parnell et al., 2012). Some of the key biological factors in development of these diseases are inflammation, oxidative stress and metabolic stress (Katta et al., 2009).

There is not much that can be done to combat aging, but some risk factors that exacerbate the negative effects of aging can be avoided. These include hypertension, obesity and smoking. The Age-Related Eye Disease Study (AREDS) reported that untreated hypertension was associated with advanced forms of AMD (AREDS, 2000). Hyman and associates went on to show that systemic hypertension with a diastolic blood pressure greater than 95mm Hg was linked to wet AMD. They found no association with dry forms (Hyman, Schachat, He, & Leske, 2000). AD studies have shown hypertension during midlife to be a risk factor for developing AD later on (Kivipelto et al., 2001). Subsequent studies have shown a protective effect of antihypertensive therapy (Haag, Hofman, Koudstaal, Breteler, & Stricker, 2009).

Some studies have shown a link between an elevated BMI (Body Mass Index) and AMD (Johnson, 2005; Katta et al., 2009). Johnson hypothesized the link could be due to the physiological changes that accompany obesity changes in lipoprotein profiles (AREDS, 2000; Johnson, 2005). Hypercholesterolemeia is a risk factor for both AD and AMD. Cholesterol affects the degradation of APP by modulating secretases. An increase in cholesterol favours beta-secretase degradation of APP into βA (Martins et al., 2009). Animal studies have shown that a diet high in fat can induce higher levels of inflammation and oxidative stress (Otaegui-Arrazola, Amiano, Elbusto, Urdaneta, & Martinez-Lage, 2013). This does not apply to all fats however, the general health benefits of omega-3 fatty acids have been known for decades (Cakiner-Egilmez, 2008). Omega-3 fatty acids contribute to the health and maintenance of many body processes. They have been associated with lower blood pressure and triglyceride levels as well as decreased βA build up (Surette, 2008). AMD studies (Cakiner-Egilmez, 2008) have shown a slower progression in vision loss with a diet high in omega-3s while observational AD studies have hypothesized this type of diet plays a neuroprotective role (Otaegui-Arrazola et al.,

2013). The definitive benefits of omega-3 fatty acids are still controversial as some studies argue otherwise (Arendash et al., 2007).

Numerous population-based studies have demonstrated the link between smoking and the increased risk of developing AMD (Delcourt, Diaz, Ponton-Sanchez, & Papoz, 1998; L V Johnson et al., 2002; Katta et al., 2009; W Smith, Mitchell, & Leeder, 1996; Velilla, García-Medina, & García-Layana, 2013; Vingerling, Hofman, Grobbee, & de Jong, 1996). Smokers have also been shown to develop AMD 5 to 10 years earlier than their non-smoking counter parts. Smoking induces a state of hypoxia. The lack of oxygen reduces the amount of antioxidants in the blood, affecting cell metabolism contributing to AD and AMD pathology (Katta et al., 2009). Smoking also exacerbates vascular injury. The nicotine from cigarettes acts on endothelial nicotinic-acetylcholine receptors (nAChR) to activate endothelial cells and augment pathological angiogenesis (Cooke & Ghebremariam, 2008; Lee & Cooke, 2012; Wu et al., 2009). This increases circulating vascular endothelial growth factor (Pons & Marin-Castano, 2011) and promotes abnormal permeability and vessel growth. Animal studies have demonstrated that the administration of nicotine accelerates the formation of choroidal neovascularization (Kiuchi et al., 2008). AD studies have found nicotine to be responsible for increased cerebrovascular permeability (Cooke & Ghebremariam, 2008). A specific nAChR, alpha7, was found to be critical in nicotine signalling and angiogenesis. This receptor is expressed on all endothelial cell types, making it a problem in both AD and AMD (Wu et al., 2009).

Histopathological Evidence

The high occurrence of comorbid AD and AMD, in addition to shared disease risk factors, suggests that these diseases may be related. This is further supported by histopathological studies showing age-related retinal changes were exaggerated in post-mortem eyes of patients with AD compared to those without (Wong et al., 2002). During the natural aging process, several pathological changes occur that affect the integrity of brain and retinal tissues. These changes are further exacerbated by lifestyle and environmental factors.

β-amyloid (βA)

The defining characteristic of both diseases is the build-up of cellular debris: senile plaques in the case of AD and drusen in AMD. Investigations into the theory of AD and AMD being related were spear-headed by the finding that β-amyloid (βA), the protein best known as a constituent of the senile plaques associated with AD, was found to be a major component of the drusen in AMD (L V Johnson et al., 2002).

βA is derived from amyloid precursor protein (APP), which has been shown to be involved in the regulation of synapse formation (Priller et al., 2006) and neural plasticity (Turner, O'Connor, Tate, & Abraham, 2003). Two alternative pathways process APP: the non-amyloidogenic pathway and the amyloidogenic pathway. The former is characterized by the cleavage of APP by the enzyme alpha-secretase. This precludes the formation of amyloidogenic peptides and leads to the release of soluble APP fragments, which have neuroprotective properties. In contrast, the latter pathway uses beta- and gamma-secretases to cleave APP into insoluble fragments, which can accumulate into plaques (Bu, 2009; Martins et al., 2009; Turner et al., 2003; Vaucher et al., 2001).

Further study into the role of βA in AMD found it was only in the drusen of AMD eyes, not the drusen associated with normal aging (Dentchev, Milam, Lee, Trojanowski, & Dunaief, 2003; Ohno-Matsui, 2011). βA immunoreactivity was found in the cytoplasm of RPE cells, particularly those located above drusen (L V Johnson et al., 2002). βA also had a greater presence in eyes with larger numbers of drusen, indicating that it may be associated with later forms of AMD (Anderson et al., 2004). Post-mortem examination of the retinas of AD patients and people suspected to have AD have found βA, but it was not found in healthy age-matched controls (Blanks, Torigoe, Hinton, & Blanks, 1996; Hinton et al., 1986).

Vesicles having layered substructures consisting of concentric ring-like elements occupy a substantial portion of drusen volume (Anderson et al., 2004). Immunoreactive staining of these vesicles was positive for βA, with the greatest concentration occurring in the outer layer (L V Johnson, 2002; Ohno-Matsui, 2011). Similar structures have been identified within the senile plaques formed in the brains of transgenic mice expressing

human APP (Terai et al., 2001). Several different types of βA assemblies have been identified in drusen. These include non-fibrillar oligomers, protofibrils and mature amyloid fibrils. Electron microscopy showed that mature fibrils are concentrated on the outside of vesicles forming a shell (Anderson et al., 2004; Isas et al., 2010).

In AD, mature fibrils are preceded by non-fibrillar βA oligomers and have distinct distributions from each other (Kayed, 2003). This was shown to be true for AMD as well when anti-oligomer antibodies were used to show accumulation of non-fibrillar βA oligomers in drusen. Their accumulation occurred close to the inner collagenous inner layer of Bruch's membrane. This proximity suggests the oligomers are formed first and interact with other proteins and lipids found in drusen to become mature fibrils. The non-fibrillar βA oligomers form distinct structures against Bruch's membrane and do not co-localize with the βA vesicular assemblies described earlier. These structures were deemed "amyloid oligomer cores". They were found not to vary in size, remaining around 15 microns. Larger drusen were found to contain multiple cores suggesting that these drusen may have formed from the coalescence of many smaller drusen (Luibl et al., 2006).

Causes of βA Production: Metal Ions

The formation of βA assemblies has been found to be an early event in both AD and AMD, but whether it is the primary event is still to be determined (Garcia-Escudero, Martin-Maestro, Perry, & Avila, 2013). Parnell and colleagues hypothesized that the accumulation of βA is due to the dyshomeostasis of metal ions (Parnell et al., 2012). APP processing shifts to the amyloidogenic pathway in the presence of metal ions due to the high affinity metal binding site on APP. When this site is occupied, the alpha-secretase binding site is blocked from starting the non-amyloidogenic pathway. Parnell's theory is supported by studies showing that βA plaques in AD contain high levels of zinc and copper. It is also worth noting that plaques are concentrated in the most zinc-rich area of the brain; the hippocampus (Frederickson & Danscher, 1990). Additionally, levels of hippocampal zinc have been shown to be higher in AD brains compared to age-matched controls (Barnham & Bush, 2008; Parnell et al., 2012). Iron has been shown to accumulate in the cortex and cerebellum of pre-clinical AD and in the RPE in AMD. This

accumulation triggers the production of superoxide anions and hydroxyl radicals, which damage brain and retinal tissue (Kaarniranta, Salminen, Haapasalo, Soininen, & Hiltunen, 2011).

Causes of βA Production: Oxidative Stress

AD and AMD share the problem of oxidative stress, another event known to occur early in both disease processes (Garcia-Escudero et al., 2013; Sivak, 2013). Oxidative stress is a pathologic mechanism common to areas of the body with high oxygen demands. It is caused by an imbalance in the generation of reactive oxygen species (ROS) and cellular ability to neutralize them or repair the damage they cause. ROS are unavoidable as they are produced as by-products from normal metabolic reactions. This causes protein misfolding and can lead to serious cellular damage (Kaarniranta et al., 2011; Katta et al., 2009).

The retina and brain are ideal for the generation of ROS due to its high oxygen consumption, the high amounts of polyunsaturated fats (PUFAs) and the regular phagocytosis of photoreceptor cell outer segments and synaptic elements. The retina has the additional detrimental factor of continuous exposure to light (Kaarniranta et al., 2011; Katta et al., 2009). The impairment in ROS clearance has been linked to mitochondrial defects. Mitochondria are critical for the maintenance of cellular integrity, energy metabolism and the regulation of ROS and apoptosis (Sivak, 2013). Dysfunction promotes ROS production starting a positive feedback loop, damaging mitochondrial DNA and oxidizing membrane lipids and proteins, which further deteriorates the organelle (Kaarniranta et al., 2011). Whether mitochondrial dysfunction is the cause or consequence of oxidative stress is still under debate.

The increased ROS-mitochondrial dysfunction is exacerbated by deficient autophagy; a process important for the quality control, cellular housekeeping and turnover of damaged or misfolded proteins (Garcia-Escudero et al., 2013). The efficiency of autophagy is also known to decrease with age. Together, declines in sufficient autophagy and mitochondrial function increase the level of ROS, which promote the accumulation of βA by facilitating the amyloidogenic degradation of APP. The presence

of βA propagates the cycle further by disrupting mitochondrial function and increasing ROS production (Kaarniranta et al., 2011; Sivak, 2013).

Causes of βA Production: Lipofuscin

The brain and retina are constantly active and have a high cellular turnover. Any dysfunction in these areas would compromise the delicate balance that exists and accelerate degeneration. Balance is disrupted when lipofuscin, consisting of cross-linked pigmentary deposits with oxidative properties, begins to accumulate. Lipofuscin is released from photoreceptor cell membranes and neurons upon their metabolism, but is indigestible by the surrounding tissue. It remains, growing in concentration with age. When enough accumulates, it causes oxidative damage to the mitochondria starting the cycle of ROS and βA discussed earlier.

Giaccone and associates (2011) put forth a new hypothesis on the origins of βA; lipofuscin. APP and βA are components of lipofuscin. The lipofuscin released into the extracellular space when neurons or RPE cells die may act as a source of βA over time (Bancher, Grundke-Iqbal, Iqbal, Kim, & Wisniewski, 1989; Giaccone, Orsi, Cupidi, & Tagliavini, 2011; Telander, 2011). Although there is no solid evidence to support this theory yet, Giaccone argues that there is also no literature to say otherwise (Giaccone et al., 2011).

Chronic Inflammation

In addition to βA, proteomic analyses have shown that the molecular components of drusen and senile plaques are similar. Among those are several complement components supporting the hypothesis of chronic inflammation contributing to the development of both diseases (Bhamra & Ashton, 2012; Hageman et al., 2001, 2005; Ohno-Matsui, 2011). Immunoreactivity studies have shown βA to co-localize with several different complement components including complement protein 3, complement factor H (CFH) and the membrane attack complex (MAC) in outer vesicular shells within drusen. This suggests a probable role for βA in the activation of the classic and alternative components of the complement system (Edwards et al., 2005; Hageman et al.,

2005; Haines et al., 2005; R Klein et al., 2005). Fewer studies have been conducted with regard to AD and the complement system, but there is support for the involvement of the classic and alternative complement pathways as well (Bhamra & Ashton, 2012). Complement components and their activation products are upregulated in AD brains, particularly in affected areas. Immunohistochemical analysis demonstrated a high incidence of MAC on dystrophic neurons, indicating an over-activation of the complement has neurotoxic consequences (Song, Poljak, Smythe, & Sachdev, 2009; Yasojima, Schwab, Mcgeer, & Mcgeer, 1999).

Microglia also play a role in chronic inflammation (Bhamra & Ashton, 2012). Active microglia have been associated with the senile plaques in AD and drusen in AMD eyes (Ohno-Matsui, 2011). Like the complement system, microglia are also activated by βA (Bhamra & Ashton, 2012). Human (Langston et al., 1999) and animal studies (P L McGeer, Schwab, Parent, & Doudet, 2003) have demonstrated that, once activated, microglia can remain active for many years after the precipitating insult. Additionally, these cells are another source of ROS. Their respiratory burst system generates super oxide ions on their external membranes, which are then released as a form of attack (E G McGeer, Klegeris, & McGeer, 2005). The activation of the complement system and microglia are intended as beneficial, part of healing. Over-activation or chronic activation, however, can lead to pathology as seen in AD and AMD.

Vascular Factors

AD and AMD have known vascular components, they even have their own vascular models (Friedman, 2004; Zlokovic, 2011), which are very similar. Both begin with the deposition of βA in capillaries, increasing the density of the microvasculature. This decreases perfusion (Berisha et al., 2007), inducing a state of hypoxia and triggering the release of vascular endothelial growth factor (VEGF) and the down regulation of an anti-angiogenic factor, pigment epithelium-derived growth factor (Ohno-Matsui, 2011; Yoshida et al., 2005). It is well known that VEGF contributes to the angiogenesis that occurs in wet AMD. In 2009, it was shown that angiogenesis is also occurring in brain regions affected by AD (Desai, Schneider, Li, Carvey, & Hendey, 2009). The decreased

perfusion also diminishes the clearance of βA. The accumulation of βA along with other cellular debris weakens the blood barriers protecting the retina and brain (Bhamra & Ashton, 2012), making them accessible to the new, leaky vessels created by angiogenesis (Biron, Dickstein, Gopaul, & Jefferies, 2011; Friedman, 2004; Vagnucci Jr. & Li, 2003; Zlokovic, 2011).

The Cholinergic System

The treatment of AD has been dominated by use of acetylcholine esterase (AChE) inhibitors. AChE is an enzyme that regulates the amount of acetylcholine (ACh) at the synapse. The cholinergic hypothesis suggests that AD is caused by a dysfunction of cholinergic neurons in the brain. Synaptic loss in AD has been shown to correlate with degree of cognitive decline (Inestrosa, Alvarez, Dinamarca, Perez-Acle, & Colombres, 2005). By inhibiting AChE, more ACh is available at the synapse for transmission. This is not the only role AChE plays in AD. It has also been shown to accelerate the assembly of beta-amyloid into plaques by acting as a chaperone (Inestrosa et al., 2005; Rees & Brimijoin, 2003). Prolonged exposure to these plaques has been shown to induce memory impairment and degeneration of cholinergic neurons in rats (Vaucher et al., 2001). A relationship between the cholinergic system and AD is well established, but its role in AMD is far less studied. Cholinergic receptors are involved in choroidal neovascularization (Kiuchi et al., 2008) and are exacerbated by smoking (Pons & Marin-Castano, 2011). The cholinergic system may also have a higher-level influence on the visual system. ACh is expressed in the visual cortex (V1). Experiments show that cholinergic reinforcement of visual stimuli induces a long-term enhancement of cortical responsiveness in V1 (visual learning) (Kang & Vaucher, 2009).

Shared Genetics

Given the structural similarities of the brain and retina as well as the common risk factors and histopathology of AD and AMD, it is not unreasonable to assume these diseases may share common genetic factors.

Genetic Contributions to AD

Approximately 5% of AD cases are classified as early onset familial AD (EOFAD). This form of AD refers to families having multiple cases occurring before the age of 60, although onset is usually in the 40s or early 50s (T D Bird, 2008). EOFAD is caused by autosomal dominant gene mutations in Presenilin-1, Presenilin-2 or Amyloid Precursor Protein. To date, over 230 mutations in these three genes alone have been shown to contribute to disease development (Qiu, Kivipelto, & Von Strauss, 2009b). These mutations affect the processing of APP causing excessive production of βA (T D Bird, 2008; Qiu et al., 2009b).

The majority of AD cases are sporadic and occur later in life. These later onset cases are thought to occur from a complex interaction of gene mutations and environmental factors. The Apolipoprotein E (ApoE) ε4 allele is known as a susceptibility gene and may contribute to familial aggregation. This means it increases the likelihood of having AD but it is neither necessary nor sufficient for development of the disease. Approximately 15-20% of cases are attributable to ApoEε4. Carriers are also more susceptible to the negative effects of excessive alcohol consumption (Qiu et al., 2009b).

When investigating gene candidates that could possibly contribute to both AD and AMD, the first to be mentioned is ApoE. ApoE is involved is lipid trafficking. It contributes to the regulation of cholesterol uptake required by cells. When cells die, they release lipids, which are bound by ApoE and redistributed to be used in cell membrane biosynthesis (Klaver et al., 1998). ApoE has been implicated in AD in numerous studies (Bu, 2009; Butterfield, 2002; Harris & Deary, 2011; Song, Poljak, Smythe, & Sachdev, 2009). It is associated with increased amounts of amyloid beta-peptides in the cerebral cortex (Patel, Adewoyin, & Chong, 2008). Similar components have been found in these cerebral cortex deposits and macular drusen. This has led to the speculation that ApoE is required to support the high rate of photoreceptor cell turnover in the macula and that impairment of its function leads to accumulation of drusen (Ding et al., 2009; Ishida et al., 2004; Klaver et al., 1998; Patel et al., 2008). This was confirmed when ApoE was

determined to be a major component of drusen (Ishida et al., 2004).

ApoE has three isoforms. The ancestral form is ApoE3. Mutations developed in ApoE3 to create the isoforms ApoE2 and ApoE4. ApoE isoforms have been shown to be associated with the development of AMD and AD through impaired processing of APP and neuronal cell membrane renewal in the brain and retina. Lipids from the degeneration of photoreceptors are not redistributed causing them to build up between the RPE and Bruch's membrane (Ding et al., 2009). A study by Patel *et al*. (2008) showed that AMD patients who were carriers of the ApoE2 variant developed the disease earlier in life compared to AMD patients that did not have this particular variant. The same study showed that the ApoE4 isoform might have protective properties due to a decreased prevalence of this isoform in patients with wet AMD (Patel et al., 2008). The opposite was found for AD. The ApoE4 confers a higher risk of developing AD while carriers of ApoE2 have a reduced risk. Despite numerous studies (Bu, 2009; Butterfield, 2002; Butterfield, Castegna, Lauderback, & Drake, 2002; Butterfield, Griffin, Munch, & Pasinetti, 2002; Logue et al., 2014; Sadigh-Eteghad et al., 2015), the explanation for the opposite allelic effects remains unclear.

Genetic Contributions to AMD

The genetic factors influencing the development of AMD have only just begun to be explored. Genetic predisposition to AMD was initially discovered through case-control association studies in the 1980s (Hyman, Lilienfeld, Ferris, & Fine, 1983). This was further supported by familial aggregation analysis (Klaver et al., 1998), segregation analysis (Yates & Moore, 2000), twin studies (Hammond et al., 2002) and classical linkage studies (Klaver et al., 1998). Higher concordance was found among monozygotic twins compared to dizygotic twins (Hammond et al., 2002). The risk of developing AMD was also shown to have an increased prevalence among first-degree relatives compared to other family members (Klaver et al., 1998).

With recent advances in gene sequencing technology and the Human Genome Project, there has been an increase in the study of single nucleotide polymorphisms

(SNPs). These are variations in DNA sequence that occur when a single nucleotide is altered. To be considered a SNP, the same variation must occur in 1% of the population. Many SNPs have no effect on protein function, but some can indicate an increased or decreased risk of developing disease. SNPs associated with a variety of pathological processes including inflammation, oxidative stress and angiogenesis have been linked to AMD (Ding et al., 2009).

Complement Factor H (CFH)

The first specific gene variant shown to be associated with AMD was the Y402H SNP in the CFH gene (Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005; R. Klein et al., 2005). It accounts for 43% of cases (Haines et al., 2005; Patel et al., 2008). The chromosomal location of CFH is 1q32. It is independently transcribed and translated in both the brain and the retina as it is too large to pass through the blood-brain or blood-retina barriers from systemic circulation (Lukiw & Alexandrov, 2012; Lukiw, Surjyadipta, Dua, & Alexandrov, 2012). Y402H refers to the amino acid substitution in the CFH protein caused by a single nucleotide change in CFH's DNA sequence, a SNP. What was normally a tyrosine (Y) amino acid was changed to a histidine (H) amino acid at position 402 in the protein. Transcripts of CFH are expressed in the RPE and choroid of the eye. Individuals with AMD have increased expression of the CFH variant in these areas compared to controls (Katta et al., 2009).

The CFH protein is involved in the alternative pathway of the complement system, the branch of the immune system responsible for chemotaxis, phagocytosis and the inflammatory response. The alternative pathway defends the body from foreign invaders such as bacteria and viruses and cleans up cell and tissue debris. This pathway must be carefully regulated to make sure only unwanted material is targeted and not the body's healthy cells. This is where CFH comes into play. It is a negative regulator of the alternative pathway, meaning it deactivates the pathway when it is not needed, protecting healthy cells from being attacked by the complement system (Ding et al., 2009; Katta et al., 2009).

The Y402H mutation is located in CFH's binding site for C-reactive protein (CRP). The SNP impairs the ability of CFH to inhibit the complement system. CRP is released into circulation in response to inflammation in the body. When CFH does not bind CRP rendering it inactive, serum levels increase in a positive feedback loop propagating the inflammatory response and causing tissue damage (Ding et al., 2009). This contributes to the generation of cellular debris and the formation of drusen (Francis & Klein, 2011).

Age-related Maculopathy Susceptibility 2 (ARMS2)

After CFHY402H, ARMS2A69S is the SNP most commonly associated with AMD. This gene is located at 10q26. Unlike CFH, ARMS2 was not a previously studied gene. It was identified from genome-wide association studies aiming to identify gene variants contributing to AMD (Fritsche et al., 2008; Jakobsdottir et al., 2005). A69S refers to an alanine to serine substitution in the amino acid sequence of the ARMS2 protein. To date, the specific function of the protein is still unknown (Kanda et al., 2007), but GWAS have determined the A69S SNP to be associated with advanced forms of AMD (Ding et al., 2009; Jakobsdottir et al., 2005).

A protein expression study by Kanda *et al*. (2007) showed that ARMS2 was localized to retinal photoreceptors. Fritsche *et al*. (2008) went on to show that the ARMS2 transcript co-localizes with a mitochondrial marker, implicating that ARMS2 is expressed in the mitochondria of the photoreceptor layer. Mitochondria are a major source of the superoxide anion, which generates hydrogen peroxide and hydroxy radicals. These components interact with DNA, protein and lipids, ultimately inducing cell death. Mitochondria have been shown to play an important role in aging and the pathogenesis of AMD. Several studies show evidence of mitochondrial alterations in AMD (Ding et al., 2009; Feher et al., 2006; Nordgaard, Karunadharma, Feng, Olsen, & Ferrington, 2008). The exact role of ARMS2 with respect to the mitochondria is unknown, but researchers hypothesize that the SNP increases oxidative stress in the retina (Ding et al., 2009; Feher et al., 2006; Fritsche et al., 2008; Jakobsdottir et al., 2005; Kanda et al., 2007; Nordgaard et al., 2008).

Are CFHY402H and ARMS2 related to AD?

In contrast to research on the AD risk gene, ApoE, with respect to its involvement in AMD, studies associating CFH and ARMS2 SNPs with AD are primitive and inconclusive. This is probably due to these SNPs being first identified in relation to AMD, facilitating further study. AD research to date has focused on the involvement of the classical complement pathway over the alternative pathway (Strohmeyer, Shen, & Rogers, 2000) due to the discovery that βA binds and activates the initiator of the classic pathway (Rogers et al., 1992). Some have noted elevated concentrations of alternative complement components in AD brains without the matching elevation in the pathway inhibitor, CFH (Emmerling, Watson, Raby, & Spiegel, 2000; Strohmeyer et al., 2000). βA is also an activator of the alternative pathway, as are many of the inflammatory processes that occur later in AD disease progression (Bradt, Kolb, & Cooper, 1998). Mutated forms of CFH were shown to occur more often in individuals with AD compared to healthy controls (Lovestone et al., 2009; Lukiw, Alexandrov, Zhao, Hill, & Bhattacharjee, 2012; Zetterberg et al., 2008). Plasma concentrations of CFH in individuals with probable AD are significantly higher than in healthy controls (Hye et al., 2006; Scholl et al., 2008; Song et al., 2009). The CFHY402H gene product was found among the proteins deposited along the surface of amyloid vesicles in the brain (Hageman et al., 2005; Ohno-Matsui, 2011). Additionally, CFHY402H has been linked to increased amounts of βA (Lukiw & Alexandrov, 2012; Lukiw, Alexandrov, et al., 2012; Lukiw, Surjyadipta, et al., 2012). One research group has been developing microRNAs to target the degradation of CFHY402H transcripts as a treatment for both AMD and AD (Lukiw, Surjyadipta, et al., 2012). Genetic linkage of the 10q26 chromosomal region, the location of ARMS2, has been associated with AD (Gatta et al., 2008). Gene expression analysis has identified transcript expression of ARMS in the cerebellum, hippocampus and cerebral cortex. Genetic studies by Gatta et al., (2008) suggest that it may infer individual risk of AD. Conversely, other studies have not found CFHY402H or ARMS2 A69S to be genetic determinants of AD (Le Fur et al., 2010; Proitsi et al., 2012; M Williams et al., 2015).

CFHY402H, ARMS2 and Drusen

The association of these SNPs has been better studied with respect to their association with drusen. CFH is synthesized by the RPE and accumulates within drusen (Hageman et al., 2005). Studies have shown that in high concentrations, the CFH protein is prone to oligomerization, facilitating the formation of drusen. The presence of the Y402H SNP increases the propotency of CFH to oligomerize (Boon et al., 2009). CFH has also been shown to affect the location of drusen. A 2015 study on individuals homozygous for the CFHY402H SNP found greater concentrations of central drusen compared to those carrying wild type CFH. The SNP was associated with drusen occupying >50% of the central 500μm radii from fovea. In addition, the same study found that CFH was associated with the progression of drusen phenotype after 2.6 years (Chang et al., 2014). This agreed with earlier studies claiming an association of CFHY402H with soft central drusen and phenotype progression (C Delcourt et al., 2011; Du et al., 2016; Magnusson et al., 2006).

ARMS2 has a smaller effect on drusen development compared to CFH. While CFH is involved in the early stages of drusen development, the role of ARMS2 is more pronounced in later stages (Dietzel et al., 2014). ARMS2 A69S is associated with a 50% risk of progression from early AMD to late and conversion of intermediate drusen to large drusen. The ARMS2S has also been associated with reticular pseudodrusen (RPD). RPD is a type of drusen recognized by the Wisconsin AMD Grading System (R Klein et al., 1991). It is described as a poorly defined network of interlacing ribbons seen via red-light fundus photography or scanning laser ophthalmoscopy (Arnold, Sarks, Killingsworth, & Sarks, 1995; R Klein et al., 1991). RPD is typically associated with a high risk of progression to late AMD. Studies linking the CFH SNP to RPD have been inconclusive with some claiming an association (Joachim, Mitchell, Rochtchina, Tan, & Wang, 2014; R J Klein et al., 2005) and others not (Boddu et al., 2014; Ueda-Arakawa et al., 2013). ARMS2 studies on the other hand, have had more definitive results (Joachim et al., 2014; Ueda-Arakawa et al., 2013; Zweifel, Imamura, Spaide, Fujiwara, & Spaide, 2010; Zweifel, Spaide, Curcio, Malek, & Imamura, 2010).

Drusen and Cognitive Impairment

Although many studies have investigated the co-occurrence of AMD and cognitive impairment, few have looked at the possible relationship between drusen deposition and cognitive status. Early studies investigated the status of the retinas of AD patients post-mortem and found increased numbers of drusen compared to retinas without the accompanying AD diagnosis (J C Blanks et al., 1996; J Blanks et al., 1996; Hinton et al., 1986). More recent studies have found cognitive impairment to be associated with dry AMD over the wet type. Dry AMD is characterized by drusen in early stages and progresses to GA in later stages (Wong et al., 2002; Woo et al., 2012). One study found that individuals who have severe cognitive impairments determined by a word-fluency test were more likely to have early AMD, soft drusen and pigmentary abnormalities compared to those without severe impairment (Wong et al., 2002). A 2012 study used a variety of global and specific cognitive tests to evaluate the status of AMD patients. Those with late AMD were found to have significantly lower scores on a word-memory test compared to those with early stages of the disease. Those with large or intermediate drusen had significantly lower scores on a digit-symbol coding task compared to those with small drusen or a normal phenotype (Lindekleiv et al., 2013).

Assessment of Visual and Cognitive Capacity

There is growing body of literature supporting a link between the presence of drusen and cognitive impairment. There is also solid evidence of the CFHY402H and ARMS2 A69S SNPs being involved in the formation and accumulation of drusen. Despite conflicting studies, this indirect evidence supports a role for these AMD SNPs in AD. The only way to gain a better understanding of AD, AMD, and their common pathogenesis is to study the spectrum of both diseases in terms of underlying genetics, abnormal brain and retinal structure and the visual and cognitive deficits. Not so long ago, this would not have been impossible, but with the sequencing of the human genome and the advent of non-invasive imaging techniques, complex diseases like AD and AMD can be studied from functional manifestation all the way across the spectrum to genotype.

Traditional Methods of Study

Retinal Function

Traditionally, visual acuity (VA) is the primary measure for evaluating the retina and subsequently, visual function. VA is a measure of the spatial resolving power of the visual system. It plays an important role in discrimination and recognition of objects and features. Hermann Snellen designed the first clinical chart for measurement of VA in 1865, but the ETDRS chart has become the universal method of measuring VA in clinical research. VA is typically measured under high contrast conditions, which is not realistic in terms of the evaluation of visual function.

It is also important to acknowledge the inaccuracy of measuring retinal function based on a single criterion. Research has shown that deficits in VA do no directly correlate with deficits in retinal/visual function nor is VA predictive of functional abilities (National Research council (US) Committee on Disability Determination for Individuals with Visual Impairments, 2002). Due to this, other aspects of vision such as contrast sensitivity and colour vision have become standard functional measures.

Contrast sensitivity (CS) can provide critical information about edges, borders and variations in luminance. Its measure can provide information on functional vision loss that is not apparent when measuring VA. It may not be helpful in terms of diagnosis, but it is a more useful measure in predicting visual function. CS seems to have a significant impact on reading ability (Leat, Legge, & Bullimore, 1999), face recognition (West et al., 2002), and mobility (Marron & Bailey, 1982). CS scores have also been correlated with subjective driving comfort (Wood & Troutbeck, 1994), crash involvement (Owsley, Stalvey, Wells, Sloane, & McGwin, 2001), and the number of “at-fault” crashes (Owsley, McGwin, & Ball, 1998) where there was no association with VA. CS is clearly more indicative of visual function than VA, but more research is required in terms of how CS can affect social participation and ability to perform everyday tasks.

Clinically, a variety of CS charts have been developed over the last few decades. The Peli-Robson Chart and/or the Mars Chart are the most widely used today. In research

situations, CS is typically measured psychophysically, using gratings over a range of spatial frequencies. This requires sophisticated equipment and programming abilities.

Colour is another measurable aspect related to retinal function. It is an important cue for identifying and distinguishing between objects. Colour deficiencies can be congenital or acquired. Congenital colour impairments are typically classified as mild or severe and are present from birth. They are typically caused by cones having altered sensitivity or by the absence of a cone type. Acquired colour deficiency is typically due to visual pathology. Most retinal disease causes impairment in the blue end of the spectrum while optic nerve pathologies lead to red-green deficits. Colour vision also changes with age. The natural yellowing of the lens over time and the development of cataracts leads to impairment in the blue end of the spectrum (National Research council (US) Committee on Disability Determination for Individuals with Visual Impairments, 2002).

A variety of colour vision tests are easily available in most clinical settings today. Pseudoisochromatic plates are used as rapid screening tools. Examples of these include the Ishihara, Dvorine and HRR plates. These tests are able to differentiate normal colour vision from different colour impairments; blue (Tritan) or red-green (duetan or protan), but do not quantify the loss. The Farnsworth-Munsell 100 Hues Test is able to classify the type of colour deficiency and the severity. It was developed from its shorter counterpart the Farnsworth-Munsell D15, which requires the arrangement of 15 different hues in a gradient from a fixed hue as a starting point.

Retinal Structure

Leeuwenhowek first studied retinal structure via microscopy in the 1600s (Masters, 1994). The knowledge gained through this method was limited because it did not allow for *in vivo* study of the retina. Microscopy is still important in the study of retinal structure today but there are other options. In the 1850s, Helmholtz developed the first ophthalmoscope, allowing clinicians to inspect the live retina, optic discs, and blood vessels for any abnormalities.

Ophthalmoscopy is still the choice method for observing the retina during routine eye examinations due to its low cost and non-invasive nature. This technique was followed by the retinal camera and fundus photography. For the first time, clinicians were able to document disease progression in their patients. Study of these physical images led to the development of the AMD grading scheme (R Klein et al., 1991). The next advancement was fluorescein angiography allowing viewing of the live retina and the integrity of retinal blood vessels (Masters, 1994). This method involves dilation of the pupils and injection of a fluorescent dye into the patient's vein. As the dye moves through the patient's body, the clinician is able to view blood vessels beneath the retinal surface that are not clearly visible otherwise. It is still used today to identify blockages or leaky retinal vessels, but is not comfortable for patients. Side effects such as an allergic reaction and nausea or vomiting can occur.

Cognitive Function

Questionnaires have been used for a long time in studies evaluating cognitive function and are used most often at present. They are readily available and extensively validated. The MMSE is the most widely used short cognitive screening test. It measures memory, language, orientation and visuoconstructive function. Although the MMSE is not sensitive to MCI (Dag et al., 2014), evidence suggests (Ala, Hughes, Kyrouac, Ghobrial, & Elble, 2002) that it may be useful in the differentiation of AD-like dementia from Lewy Body Dementia. The MoCA, although less widely used, is sensitive to MCI. It measures visuospatial capabilities, language, memory, executive function, attention and orientation. Compared to the MMSE, the MoCA has a sensitivity of 90% versus 18% in detecting MCI (Dag et al., 2014). The MMSE and MoCA both have "Blind" versions validated for use in visually impaired populations (Busse, Sonntag, Bischkopf, Matschinger, & Angermeyer, 2002; Wittich, Phillips, Nasreddine, & Chertkow, 2010).

Newer Methods of Study

Retinal Structure: Optical Coherence Tomography (OCT)

The retina is composed of several functional layers and in order to truly study retinal structure, tomographic viewing is necessary. This was made possible by the

development of optical coherence tomography (OCT) in the 1990s. This was the first non-invasive, non-contact instrument allowing cross-sectional imaging of the live retina. The OCT interprets the interference pattern created from backscattering light reflected from the retinal layers and a reference mirror. Users were able to visualize and measure the thickness of retinal layers in addition to detecting swelling, fluid and abnormal tissue. Over the last several years, research has begun to investigate on the characteristics of drusen such as contour, reflectivity, and content that can be identified on OCT images with the hope of developing a method of predicting progression to later stages of AMD (Khanifar et al., 2010; Leuschen et al., 2013; Schlanitz et al., 2015). The relationships between these characteristics and their impact if any on visual function have yet to be explored.

Each generation of OCT improved in terms of scan speed and image resolution. Eventually, OCT was combined with scanning laser ophthalmoscopy (SLO), enabling a fundus image and a cross-sectional image of the retina to be taken simultaneously. The OCT and SLO images can be correlated pixel to pixel to generate 3D topographical maps, providing more information on the retina than ever before.

Retinal Function: Scanning Laser Ophthalmoscope (SLO)

In the laboratory, the evaluation of visual function improved and accelerated by the development of the Scanning Laser Ophthalmoscope (SLO) in the early 1980s. The SLO allows the monitoring of dynamic processes such as blood flow and fixation stability (Reinholz, Ashman, & Eikelboom, 1999) as well as the assessment of retinal function and visual behaviour. Visual stimuli can be projected onto the retina while it is simultaneously viewed, the retina enabling observers to see what part of the retina an individual is using to view a stimulus (Seiple, Rosen, & Garcia, 2013).

Webb and colleagues introduced the first SLO model, the Flying TV Ophthalmoscope, in 1980. Its purpose was to make viewing the fundus for an extended period of time more comfortable for the patient (Webb, Hughes, & Pomerantzeff, 1980). It also enabled observation for multiple viewers, proving useful for education and consultation (Webb, 1983). The perimetry feature was added shortly after. Timberlake

demonstrated the usefulness of the SLO in measuring visual function in AMD by its ability to map the retinal location of scotomata (Timberlake, Mainster, Webb, Hughes, & Trempe, 1982). By the 1990s, the first SLOs were commercialized and had begun to be paired with other well-known techniques including electroretinography, angiography, Doppler flowmetry and optical coherence tomography (OCT).

The next generation SLOs had the option of imaging at several wavelengths simultaneously (Reinholz et al., 1999) or sequentially (Manivannan et al., 2001). This increased resolution and allowed the added features of eye-tracking, adaptive optics and combination OCT/SLO (Sharp, Manivannan, Xu, & Forrester, 2004). Eye motion was a major problem for both diagnostic and therapeutic treatments such as laser photocoagulation. Hammer *et al*. reported the use of an integrated SLO and retinal tracker in 2003. A tracking beam was used to lock onto a retinal feature and sense the motion of the eye. The information from the tracking beam movement was used to steer imaging (Hammer et al., 2003). MacKeben *et al.* improved upon this with the development of "smart microperimetry". The program provides a gaze-contingent display of the stimulus and senses the conditions for image tracking so that stimulus position can be corrected in the case of blinks and involuntary eye movements (MacKeben & Gofen, 2007). This improved the mapping of scotomata and the imaging of patients with fixation problems such as those with diabetic retinopathy and AMD (Hammer et al., 2003; Sharp et al., 2004). In 2002, Roorda *et al.*, described the first SLO to use adaptive optics. This improved the resolution of the SLO such that, for the first time, it was possible to visualize photoreceptors, nerve fibres and the flow of white blood cells in retinal capillaries (Roorda et al., 2002; Sharp et al., 2004). The combination OCT/SLO allows the exact location of the OCT line-scan to be seen on the SLO image. This is helpful for locating the anatomical fovea when it is damaged in the case of disorders like macular hole, AMD or diabetic retinopathy (Sharp et al., 2004).

Eccentric viewing is a common strategy adapted by individuals with visual impairment, but is not typically part of the equation in evaluating visual function. It involves directing the eye so that an image falls on healthy portions of the retina. In the 1980s, Timberlake coined the term "Preferred Retinal Locus" or PRL to identify this

region. Much investigation has been devoted to the variable characteristics of a PRL. Thanks to numerous SLO studies (Crossland, Culham, Kabanarou, & Rubin, 2005; Crossland, Engel, & Legge, 2011; Deruaz, Whatham, Mermoud, & Safran, 2002; Fletcher & Schuchard, 1997; Fletcher, Schuchard, & Watson, 1999; Greenstein et al., 2008; Guez, Le Gargasson, Rigaudiere, & O'Regan, 1993; Lei & Schuchard, 1997; Nilsson, Frennesson, & Nilsson, 1998, 2003; Schuchard, 2005; Timberlake et al., 2005; Timberlake, Bothwell, & Moyer, 2013; Timberlake, Omoscharka, Grose, & Bothwell, 2012; Watson, Schuchard, De l'Aune, & Watkins, 2006), we know that a PRL is a discrete, well-defined region where fixation occurs. It may be task-specific and is repeatable within and between trials. The PRL is not always the best region of the retina to be used, but it is the habitual region (Crossland et al., 2011).

A successful PRL allows an individual to scan and steadily fixate on a target in order to view details as the fovea would (Crossland, Culham, & Rubin, 2004; R W Cummings, Whittaker, Watson, & Budd, 1985; Schuchard, 2005). The SLO allows the quantitative measure of a PRL through fixation stability. The participant is asked to view a target for 20 seconds maintaining gaze as steady as possible. The SLO records the position of gaze with respect to the target while correcting for eye movements based on retinal anatomy. These positions are captured at regular intervals over the duration of the test resulting in an image of the fixating positions superimposed over the target. The quantitative measure is the bivariate contour ellipse area (BCEA) (Steinman, 1965), which is an ellipse encompassing the target and the majority of fixation positions. A large BCEA indicates poor fixation stability, while a smaller one indicates stable fixation.

There has been a plethora of research with regards to the PRL and various functional tasks including reading (Cheung & Legge, 2005; Crossland et al., 2004; Culham, Fitzke, Timberlake, & Marshall, 1992; R W Cummings et al., 1985; Fletcher et al., 1999; Kabanarou & Rubin, 2006; Nilsson et al., 1998, 2003; Palmer, Logan, Nabili, & Dutton, 2010; Seiple, Szlyk, McMahon, Pulido, & Fishman, 2005; Seiple, Grant, & Szlyk, 2011; Timberlake et al., 1986; Watson et al., 2006), handwriting (Timberlake et al., 2013), face recognition (Seiple et al., 2013) and reach-grasp tasks (Timberlake, Grose, Quaney, & Maino, 2008; Timberlake et al., 2012; Timberlake, Omoscharka, Quaney,

Grose, & Maino, 2011). This research has led to eccentric viewing training and the development of a PRL for reading at least, to be common rehabilitation strategies.

Cognitive Function

Where the imaging of retinal structure and the evaluation of visual function have made huge advances, imaging of the brain and its activity has not come as far. Functional magnetic resonance imaging (fMRI) is the latest in a long line of tools that uses blood flow and oxygenation to infer brain activity. It was developed in the 1990s and relies on the concept that when a brain area is more active, it requires more oxygen and blood flow to that particular area in order to meet the higher demands. Based on this, activation maps can be created based on which areas of the brain are required to carry out certain mental processes. The use of fMRI to image the brain is attractive due to the high-resolution images it can produce and the fact that it is relatively simple to use. It has the added benefits of being non-invasive and not reliant on radiation making it safe for patients. Where fMRI falls short is in validating its results (Eklund, Nichols, & Knutsson, 2016; Etz & Vandekerckhove, 2016; Open Science Collaboration, 2015). The cost of using fMRI is so high that studies are limited to small sample sizes and very few labs are able to afford running repeat experiments to replicate results. Moreover, a recent study has brought into question the validity of the software being used to interpret fMRI results. Some of the most popular software packages for analysis of fMRI images resulted in false-positive rates of up to 70%. This study has brought into question some 400,000 studies published in reputable journals since 1992 (Eklund et al., 2016).

Now that computer programming is a more widely available skill, psychophysical testing of cognition is becoming more mainstream and is useful in combination with cognitive screening questionnaires. The Neurominder by Cognisens measures mild perceptual impairment, the precursor to cognitive impairment. It examines perceptual cognitive skills such as perceptual processing and working memory capacity for visual stimuli. It has been used to evaluate second-order perceptual processing in athletes and older adults compared to younger adults (Faubert, 2002; Faubert & Sidebottom, 2012; Legault & Faubert, 2012). Deficits in second-order processing are thought to be one of

the initial signs of MCI (Faubert, 2002; Sara & Faubert, 2000; Tang & Zhou, 2009). These deficits are so subtle, they are unlikely to be identified by traditional questionnaires.

Psychophysics can also be used to measure higher-order cognitive function. For example, a Neurotracker also by Cognisens, requires participants to follow targets through dynamic motion across a wide 3D projection (Legault & Faubert, 2012). Based on fMRI studies, the areas of the brain activated by neurotracking have been identified. Some of these areas are responsible for the eye movements required during 3D multiple object tracking (3DMOT). These are the superior parietal lobule and the frontal and supplementary eye fields. Higher-order brain areas involved in processing visual information are also activated. These involve attention, which is a prerequisite for forming memory. The human motion area or V5 is activated. This area of the brain is sensitive to motion and is retinotopically organized. The anterior and posterior intraparietal sulci are also activated. These areas are responsible for deciphering object location and features, respectively (Howe, Horowitz, Morocz, Wolfe, & Livingstone, 2009; Jain et al., 2010). The combination of cognitive questionnaires and psychophysical tools available today allows the exploration of cognitive function on a continuum.

Objectives

A number of studies have reported an association between AMD and impaired cognitive function. However, these studies have been limited with regard to the level of evaluation of the retina. Many of these studies were based on diagnosis alone and did not examine the status of the retina. Those that did evaluate the retina were limited to broad classifications by using only fundus photography. To date, no study has investigated the link between the extent, location and characteristics of drusenoid deposits in AMD and the presence of cognitive impairment. Moreover, there is no literature on any genetic associations with these drusenoid components. Retinotopic quantification of drusen is made possible by newer technologies such as the OCT/SLO unit.

Cognitive assessment of participants in comorbid AMD-AD studies has also been limited. Several studies have relied on subjective memory complaints, which have been

shown to be inaccurate compared to more objective measures (Clement, Gauthier, & Belleville, 2012; Flicker, Ferris, & Reisberg, 1993; G E Smith, Petersen, Ivnik, Malec, & Tangalos, 1996). Others have used subscales of neuropsychological tests to assess cognitive function. Subscales by themselves cannot be used to reach a clinical diagnosis of cognitive impairment. Cognitive screening tools such as the MMSE have shown cognitive impairment to be associated with late AMD, but not earlier stages. Given research showing the MMSE is not sensitive to MCI (Dag et al., 2014), it is possible that AMD is associated with cognitive deficits too mild to be detected.

The ocular manifestations of AD have been well documented, but retinal characteristics predictive of cognitive deterioration, if any, have not yet been established.

The major objective of this study was to explore the genotype-structure-function relationship in AMD and MCI. The genetic focus was the SNPs CFHY402H and ARMSA69S whose contribution to the cognitive side of this equation is still not well understood. Retinal structure and retinal/cognitive function were evaluated using the traditional methods outlined above, but also combined with the more sophisticated technologies that have become available more recently.

This study has:

- Sought to support the findings of previous studies showing that carriers of the SNPs, CFHY402H and ARMS2A69S are more likely to have AMD than non-carriers.
- Further explored the connection between the SNPs of interest and the functional consequences of AMD as measured by traditional ophthalmologic tests and SLO.
- Utilized OCT to investigate a possible correlation between drusen characteristics and the SNPs of interest.
- Attempted to replicate recent studies demonstrating a higher prevalence of cognitive impairment among individuals with AMD compared to age-matched controls using traditional questionnaires and more sophisticated psychophysical measures.

- Investigated whether cognitive impairment can be linked to the presence of AMD SNPs.

The following hypotheses were examined:

- Carriers of the CFHY402H and ARMS2A69S SNPs would be more likely to have AMD than non-carriers.
- Carriers of the SNPs of interest would have greater functional impairment compared to non-carriers.
- Drusen characteristics as seen via OCT, such as drusen with core and hyperreflective foci above drusen, would be indicative of later stages of AMD.
- Individuals with AMD would score lower on cognitive measures compared to age-matched controls
- Carriers of the AMD SNPs would have poorer cognitive performance than non-carriers.

Chapter 2: Study 1 - Genotyping

Age-related Macular Degeneration (AMD) is a complex disease whose development is influenced by a variety of genetic, environmental and lifestyle factors. Certain lifestyle factors that contribute to AMD such as diet and sun exposure can be controlled. It is important to study genetics given that it is a contributing factor which, to date, cannot be controlled. Understanding the molecular mechanisms involved in AMD while taking into account gene expression and epigenetic factors and how they interact with lifestyle factors will be important in developing therapeutic strategies.

Genetic predisposition to AMD was initially discovered in the 1980s through case-control studies (Hyman et al., 1983). Initially, no genetic loci were identified and the strength of the heritability of AMD was questioned due to failure of the disease to follow traditional Mendelian patterns. Eventually, epidemiological and genetic studies established the genetic factor to have 45-70% contribution to disease development (Seddon, Cote, Page, Aggen, & Neale, 2005). Higher concordance was found among monozygotic twins compared to dizygotic twins (Hammond et al., 2002; Klaver et al., 1998; M L Klein, Mauldin, & Stoumbos, 1994; Seddon et al., 2005). This was further supported by other familial studies (Klaver et al., 1998; W Smith & Mitchell, 1998).

Prior to the genetic breakthrough that was the Human Genome Project, several studies investigating the pathogenesis of AMD established that several inflammatory and immunologic mediators were involved (Anderson, Mullins, Hageman, & Johnson, 2002; Anderson et al., 2010; Ding et al., 2009; Patel et al., 2008). Early genetic linkage analyses on siblings and multiplex families suggested the involvement of the *ABCA4* locus, identified as causing autosomal recessive Stargardt disease (Allikmets, Singh, et al., 1997) to be linked to AMD as well, but reports were inconsistent (Allikmets, 2000; Allikmets, Shroyer, et al., 1997; Guymer, 2001; Shroyer, Lewis, Yatsenko, Wensel, & Lupski, 2001). Finally, a large meta-analysis of all these genetic linkage studies convincingly revealed chromosomal regions 1q23.3-q32 and 10q26 to harbour AMD loci (Fisher et al., 2005).

With the continued progress from the Human Genome Project, single nucleotide polymorphisms (SNPs) were identified and new methods of identifying AMD loci became available. Four independent research groups using complimentary genetics approaches converged on the association of AMD with a SNP in the gene encoding complement factor H on chromosome 1q32 (Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005; R J Klein et al., 2005). This discovery was not only a major landmark in the genetics of a complex disease, but it was also the first validation of the Genome-wide Association Study (GWAS) approach. Since this pivotal breakthrough, GWAS has been used to investigate numerous genetic diseases, resulting in over 2000 publications (Cooke Bailey, Pericak-Vance, & Haines, 2014; Welter et al., 2014). GWAS studies in AMD now boast over one million markers (Neale et al., 2010), including the locus with the strongest single genetic effect; ARMS2A69S on chromosome q26 (Jakobsdottir et al., 2005; Schwartz et al., 2014).

The purpose of this first study is to show the genetic distribution of the two SNPs with the strongest genetic effect on AMD in this study population. It was expected that the distribution would agree with countless studies showing that CFHY402H and ARMS2 contribute to over 50% of AMD cases.

Methods

Phase I

Phase I of this investigation involved participants from a previous study (Smailhodzic et al., 2012) who had been genotyped from blood samples by Radboud University Medical Centre in Nijmegen, Netherlands. Participants were genotyped from blood samples for the following SNPs: rs4986790, rs10490924 (ARMS2A69S), rs2511989, rs2230199, rs1800555, rs1800553, rs3775291, rs4151667, rs429358, rs7412, rs10033900, rs10468017, rs9621532, rs1410996, rs9332739, rs699946, rs12678919, rs1883025, rs17457, rs3764261 and rs1061170 (CFHY402H). The study protocol was approved by the McGill University Health Centre research ethics committee and followed

the tenets of the Declaration of Helsinki. All study participants gave signed, informed consent prior to their participation.

From the pool of previously genotyped participants, all were recruited from the Montreal Retina Institute. The current study protocol was approved by *Le Comité d'éthique de la recherche en santé* at the Université de Montréal and followed the tenets of the Declaration of Helsinki. All study participants gave signed, informed consent prior to their participation in the study (Appendix A). Individuals aged 70 years or older with a diagnosis of AMD were included in the current study. Subjects with comorbid glaucoma or other retinal disorders were excluded. Subjects with diagnosed dementia or neurological impairments were also excluded.

Phase II

This phase of the study expanded the subject pool by recruiting more AMD subjects and adding a control group. The results from Phase I determined the SNPs used in the genetic testing conducted in Phase II.

Participants were recruited via "word of mouth" and from the School of Optometry Clinic at the Université de Montréal. Study protocol was approved by *Le Comité d'éthique de la recherche en santé* at the Université de Montréal and followed the tenets of the Declaration of Helsinki. All study participants gave signed informed consent prior to their participation in the study.

AMD-subject characteristics were the same as those in Phase I. The control group also consisted of participants aged 70 years or older. They were required to have normal, healthy retinas. Exclusion criteria included retinal disease, glaucoma and diagnosed cognitive problems.

Asper Biotech Ltd. in Estonia conducted genotyping from saliva samples. Their AMD program uses targeted mutation analysis to identify three SNPs: rs1061170, rs10490924 and rs1410996. Based on the results obtained during Phase I, the SNP rs17457 was added to the panel.

Phase I: Results and Discussion

Results

A total of 107 individuals were genotyped from the previous study (Smailhodzic et al., 2012). Mortality or development of AD since genotyping excluded 15 potential participants. Six were unreachable, two were excluded because they were under 70 years of age and 74 declined further participation. Ten individuals (3M, 7F) agreed to participate in more testing. Their characteristics can be seen in Table I.

Two participants had dry AMD in one eye and wet AMD in the other. The remaining eight participants had wet AMD in both eyes. There were four participants who carried neither of the ARMS2 or the CFH SNPs. The ARMS2 SNP was carried by 6/10 participants with half of them being homozygous for the mutation. Only three individuals carried the CFH SNP, with one being homozygous. Three individuals carried both SNPs of interest.

The other SNPs tested as part of the AMD panel were not of interest in this study, but it was noted that 9/10 participants were carriers of a SNP, rs174547, from Fatty Acid Desaturase 1 (FADS1). Of these individuals, 6 were homozygous.

Table I. Phase I Participant Characteristics

ID	**Sex**	**Age**	**AMD Diagnosis**		**CFHY402H***	**ARMS2A69S***
			OD	*OS*		
AMD1	F	80	Wet	Wet	0	2
AMD2	F	82	Wet	Wet	1	1
AMD3	M	72	Wet	Wet	0	0
AMD4	F	79	Wet	Wet	2	2
AMD5	F	86	Wet	Wet	1	1
AMD6	F	75	Wet	Wet	0	0
AMD7	F	85	Wet	Wet	0	0
AMD9	M	92	Dry	Wet	0	1
AMD10	F	79	Wet	Wet	0	2
AMD11	M	84	Wet	Dry	0	0

*The numbers in these columns represent the number of copies of the SNP the participant has.

Discussion

Given that the participants were recruited from retinal specialists, the majority of them had wet AMD. Currently, retinal specialists treat only wet AMD on a regular basis. The majority of those with dry AMD remain in the care of general ophthalmologists or optometrists for monitoring purposes.

As expected, approximately half of the participants were carriers of the ARMS2 SNP. Only three individuals carried the CFH SNP. This was an unexpected finding given that CFHY402H is said to account for approximately 43% of AMD cases (Haines et al., 2005). Even more surprising was that the majority of the sample members were carriers of the FADS1 SNP. FADS1 has been linked to AMD through GWAS studies (Neale et al., 2010) and has been shown to be a genetic factor in heart disease, contributing particularly to plaque build-up (Lettre et al., 2011; Merino et al., 2011; Merino, Ma, & Mutch, 2010; Park, Kim, Lee, & Park, 2011) . Both of these anomalies could possibly be explained by the small sample of individuals who agreed to further testing. To determine this, the genetic results of the original 107 were analysed.

In the original sample, 69 individuals (64.5%) were carriers of the CFHY402H SNP and of them, 36.2% were homozygous. These results were in better agreement with previous findings concerning the contribution of the CFH SNP to AMD (Haines et al., 2005), confirming that the small sample size was the problem.

The original sample also contained 79 individuals (73.8%) who were carriers of the ARMSA69S SNP and, of them, 32.9% were homozygous. There were 56 individuals carrying copies of both SNPs of interest with 7 of them being homozygous for both. Eight individuals carried no copies of either SNP.

In terms of the FADS1 SNP, 91 individuals (85%) were found to be carriers and of them, 53.9% were homozygous.

Phase II: Results and Discussion

Results

A total of 31 individuals were genotyped as part of Phase II. Two participants were excluded due to co-morbidities. There were 11 participants with AMD (1M, 10F) having an age range of 71-87 years. These results were combined with those of Phase I to form the final AMD group (N=21). The control group consisted of 18 individuals (6M, 12F) with an age range of 70-85 years. The genotyping results of Phases I and II were combined and are shown in Tables II and III.

The CFHY402H SNP was found in 21/39 (53.8%) participants. Of the AMD group, 10 individuals were carriers, with five being homozygous for the SNP. There were 11 carriers in the control group, with only one of them being homozygous.

The ARMS2A69S SNP was also found in 21/39 (53.8%) participants. Of the AMD group, 12 were carriers with five being homozygous for the SNP. There were nine carriers in the control group with only one being homozygous.

The FADS1 SNP was found in 33/39 (84.6%) participants. Of the AMD group, 17 were carriers with nine being homozygous for the SNP. There were 16 carriers in the control group, with six being homozygous.

Discussion

After recruitment and testing in Phase II, the distribution of carriers of the CFH and ARMS2 SNPs was as expected for the AMD group. Homozygotes of either SNP were more likely to be in the AMD group than in the control group. The surprising result was the high frequency of carriers of the FADS1 rs174547 T allele.

Table II. Participant Characteristics: AMD v. Control Groups

Group	**N**	**Sex**		**Age**	**Diagnosis**		
		M	*F*		*Wet*	*Dry*	*Both*
AMD	21	4	17	78.14 ± 6.71	11	5	5
Control	18	6	12	74.00 ± 3.86	na	na	na

na: not applicable

Table III. Genetic Results

SNP	**AMD**	**Control**	**Total**
CFHY402H	10	11	21
homozygotes	5	1	6
ARMS2A69S	12	9	21
homozygotes	5	1	6
FADS1	17	16	33
homozygotes	9	6	15

FADS1 Gene Product and its Function

FADS1 encodes an enzyme involved in lipid metabolism, one of the three pathogenic systems identified as carrying genetic mutations that contribute to AMD (Fritsche et al., 2014). More specifically, the FADS1 gene encodes delta-5 fatty acid desaturase, the rate-limiting enzyme required for polyunsaturated fatty acid (PUFA) biosynthesis (Figure 1) (Dumont et al., 2011; Martinelli et al., 2008). This desaturase is involved in both the omega-6 and omega-3 pathways. PUFAs are essential in regulating cellular membrane fluidity, intracellular signalling and transcriptional regulation (Jung, Torrejon, Tighe, & Deckelbaum, 2008). The SNP, FADS1 rs174547, was first linked to AMD in GWAS studies (Neale et al., 2010). This was followed by candidate gene studies (Fauser et al., 2011; Gorin, 2012; Lechanteur et al., 2012; Merle et al., 2011). These research groups suggested that changes in the metabolism of high-density lipoprotein (HDL) cholesterol play a role in AMD, possibly through the accumulation of lipids and cholesterol in drusen. Studies that followed this line of thought by investigating serum HDL levels with respect to AMD have created confusion. Results have been conflicting with some research showing no association between AMD and circulating levels of HDL (Abalain et al., 2002), others have confirmed GWAS theories of AMD being linked to higher levels of HDL (C Delcourt et al., 2001; van Leeuwen et al., 2004), while the inverse has been found for other groups (Wachter et al., 2004).

The alleles present at rs174547 are C, the ancestral allele, or T. The T allele is considered the risk allele for AMD. The SNP has been associated with altered desaturase activity, and omega-3 and omega-6 PUFA biosynthesis. The presence of the T allele results in higher delta-5 fatty acid desaturase activity and higher levels of circulating HDL (Fauser et al., 2011; Hellstrand et al., 2012; Merino et al., 2011). The increased levels of HDL contribute to drusen formation as mentioned earlier, but the risk contributed by the increase in delta-5 fatty acid desaturase activity could be two-fold. Firstly, the omega-3 and omega-6 PUFA biosynthesis pathways compete for use of the delta-5 enzyme, with the omega-6 pathway usually coming out on top. This means that there is always a lower ratio of omega-3 fatty acids compared to omega-6. Due to the

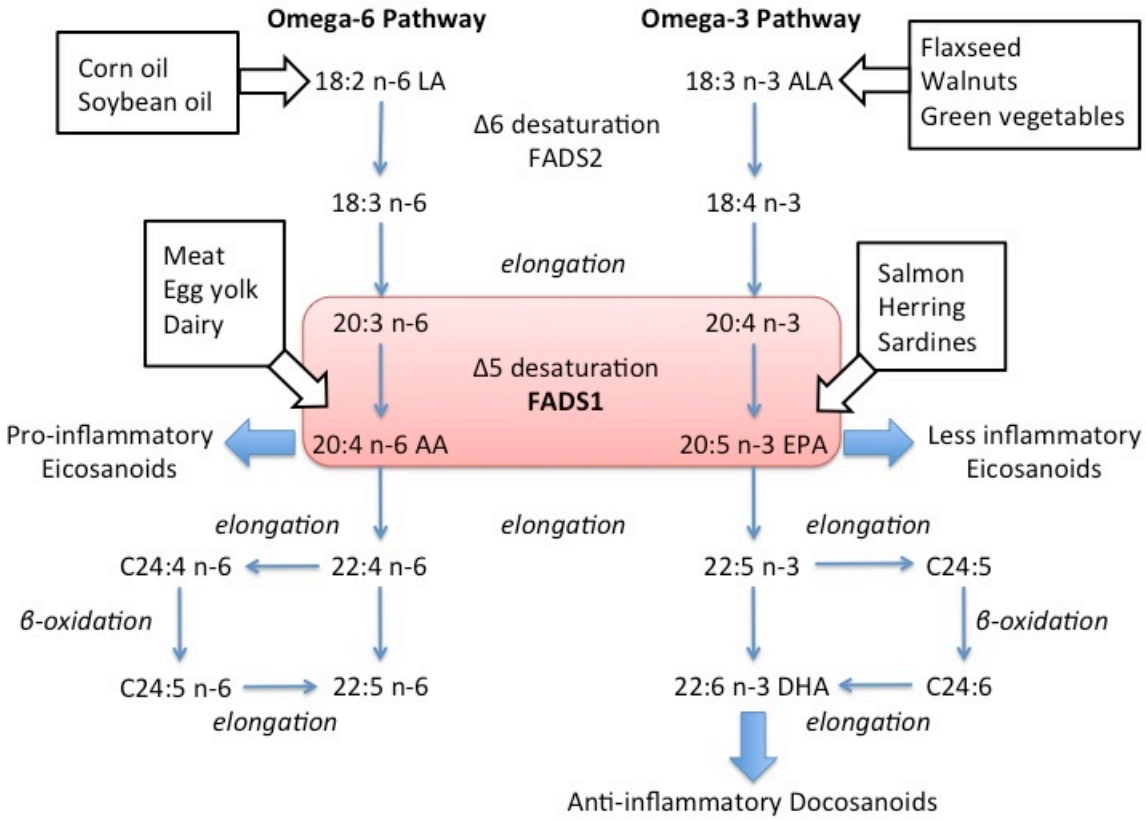

Figure 1. The FADS1 Pathway.

The FADS1 gene encodes the enzyme Δ5-desaturase, which plays a role in omega-6 and omega-3 fatty acid biosynthesis. The enzyme catalyzes the step required to produce arachidonic acid and eicopentaenoic acid (EPA), which the body uses to produce pro-inflammatory and anti-inflammatory eicosanoids respectively. EPA can be converted into docosohexaenoic acid (DHA), which is used to produce stronger anti-inflammatory molecules.

LA: Linoleic acid, ALA: α-Linolenic acid, AA: Arachidonic acid, EPA: Eicopentaenoic acid, DHA: Docosohexaenoic acid

western diet, the ratio of circulating omega-3 fatty acids to omega-6 is approximately 1:10-20 (Cakiner-Egilmez, 2008). The retina has high concentrations of docosahexanoic acid (DHA) and eicosapentanoic acid (EPA), two long chain omega-3 fatty acids (Augood et al., 2008). Omega-3s from all sources are incorporated into phospholipid bilayer of cells in all body tissues. There, they are able to interact with membrane receptors to alter transduction pathways, inflammation, angiogenesis and affect cell processes such as apoptosis and cell survival. Some tissues such as the retina, brain and myocardium are particularly enriched. Changes in omega-3 levels can be measured only days after increasing dietary intake in these tissues (Surette, 2008). Diet-induced deficiencies of omega-3s have been known to alter photoreceptor function (Cakiner-Egilmez, 2008). Retinal function depends on an adequate amount of DHA. Approximately 50% of the lipids in photoreceptor rod outer segments are DHA (Tuo et al., 2009).

Secondly, omega-6 PUFAs are used to synthesize eicosanoids, signalling molecules, which include prostiglandins, leukotrienes and thromboxane (Serini, Fasano, Piccioni, Cittadini, & Calviello, 2011). These molecules are released in response to injury and their action is required to help in the repair of damaged tissue. They play an important role in the inflammatory pathway, which has been shown to be involved in the pathology of AMD (Cakiner-Egilmez, 2008; Katta et al., 2009). Additionally, omega-6 PUFAs compete with omega-3s for incorporation into cell membranes. The presence of omega-3s in cell membranes serves to dampen the inflammatory response, but without enough of them, the inflammation brought about by high levels of omega-6 PUFAs can go unchecked (Cakiner-Egilmez, 2008; Serini et al., 2011).

FADS1 rs174547 Epidemiology

The major allele at this location differs depending on the population. The T allele is the minor allele in Mexican (0.39), Native American (0.21) and Native Hawaiian (0.42) populations, while it is the major allele in European (0.66), African (0.91) and Japanese (0.59) populations. This difference across populations coincides with AMD risk. Individuals of European, African and Japanese decent are at a higher risk of developing

AMD compared to those of Mexican, Native American or Native Hawaiian descent (Dumitrescu et al., 2011).

The population of the current study, consisting mostly of individuals of French-Canadian heritage, could be considered most similar to a European population, or an American population. According to the dbSNP, a database of genetic and epidemiological information on SNPs from the National Institute of Health, the frequency for the T allele of rs174547 in an American population is lower than that of a European population, at 0.41 (NIH, 2017). The frequency of the T allele in this population is 0.85. This is greater than that of either the European or American frequencies.

This could be due to the fact that the Quebec population is a known Founder population (Roy-Gagnon et al., 2011). A Founder population is a new population that is established from very few individuals (or founders) and, as a result, exhibits reduced genetic variation. Due to this, rare disease alleles are enriched, leading to higher numbers of homozygotes displaying the disease phenotype (Kristiansson, Naukkarinen, & Peltonen, 2008). Such populations have been instrumental in medical genetics for research on genetic diseases. The Quebec population has been valuable in the study of genotype-phenotype interactions in Usher syndrome (Ebermann et al., 2009) and retinitis pigmentosa (Coussa et al., 2015; Koenekoop et al., 2003). The Founder Effect could potentially explain the increased frequency of the T allele at rs17457 in this study population.

Summary and Conclusion

Genetic studies of complex disease have recently become possible, but they have required vast study cohorts for an individual trait and international collaborations on enormous scales (Consortium, 2007). Large global populations may not always be necessary to study the genetics of complex diseases, like AMD. Susceptibility to complex disease involves contributions from common variants and rare variants. Several common variants are likely to explain a substantial fraction of the genetic contribution to a complex disease, while more rare variants have a greater impact on the phenotype of the

disease. The statistical power required to detect susceptibility alleles is positively correlated with the frequency of the allele and the penetrance, or degree of phenotypic expression of the allele in the test population. Founder populations may be required to better define a risk allele that, although significant, gets lost in GWAS as a result of population-specific effects. A number of researchers have discussed the advantages of the use of Founder populations in medical genetics. Some of the benefits include genetic, environmental and phenotypic homogeneity, good genealogical records, higher degree of linkage disequilibrium, and reduced allelic heterogeneity (Cohen et al., 2004; Kristiansson et al., 2008; Lohmueller, Pearce, Pike, Lander, & Hirschhorn, 2003; Zeggini et al., 2005).

This study has shown that the most influential SNPs in AMD occur as expected in the Quebec population, with just over 50% of the study sample carrying CFHY402H and ARMS2A69S. The Quebec population has also been potentially identified as having a Founder Effect for FADS1 rs17457. This SNP has been identified as a significant contributor to AMD in GWAS studies (Neale et al., 2010), but its role has not been well characterized. Due to its high frequency, the Quebec population is an ideal sample to achieve a better understanding of the role that the FADS1 SNP plays in the leading cause of legal blindness in the Western world.

Chapter 3: Study 2 - Visual Function

Hutchison and Tay were the first to describe Age-related Macular Degeneration (AMD) as "symmetrical central choroidoretinal disease occurring in senile persons" (Hutchinson & Tay, 1875). Today, AMD is known as a complex, late-onset retinal disease characterized by the progressive and irreversible loss of central vision affecting the macula. It is the leading cause of blindness in older adults (Bergeron-Sawitzke et al., 2009; Gehrs, Anderson, Johnson, & Hageman, 2006; Katta et al., 2009).

Through traditional methods of evaluating visual function, the deficits caused by the disease are well characterized. Eye-chart tests have shown that individuals with AMD suffer losses in visual acuity (Alexander et al., 1988; Cacho, Dickinson, Reeves, & Harper, 2007). These losses may be gradual in the case of atrophic or dry AMD or sudden in the case of exudative or wet AMD. Losses in contrast sensitivity have also been documented, using a series of gratings varying in spatial frequency and contrast or letters of decreasing contrast (Lennerstrand & Ahlstrom, 1989; Midena, Angeli, Blarzino, Valenti, & Segato, 1997). Visual field assessments have demonstrated that most AMD patients lose visual function in some region of their macula. Stereovision tests, such as the Titmus and the Randot, have determined that those with AMD have poor stereopsis (Cao & Markowitz, 2014). These losses are common to all individuals who develop AMD, but their degree of severity and progression is not. Individuals who have had the same subtype of AMD (wet or dry) for the same length of time will rarely display functional losses with the same characteristics (Fletcher & Schuchard, 1997; Rees, Kabanarou, Culham, & Rubin, 2005).

Single nucleotide polymorphisms (SNPs) play a role in the development of AMD. They have been shown to contribute to disease incidence (Andreoli et al., 2009; Johanna M. Seddon et al., 2009), progression (Dietzel et al., 2014) and subtype (Andreoli et al., 2009; Cheng et al., 2013; Wegscheider et al., 2007). Since the post-Human Genome Project era, molecular biologists have been trying to establish how each AMD SNP affects the function of its respective protein product and the mechanism by which it contributes to retinal degeneration (Cooke Bailey et al., 2014). More recently, SNPs have

been studied with respect to their roles in treatment response to anti-VEGF (Imai et al., 2010; Lee, Raya, Kymes, Shiels, & Brantley Jr., 2009; Riaz et al., 2016; Smailhodzic et al., 2012) and mineral supplements (Awh, Lane, Hawken, Zanke, & Kim, 2013; Chew et al., 2014; B. M. J. Merle et al., 2015), but they have not been well studied in terms of their association with visual function. With genotyping becoming more common in the monitoring and treatment of AMD, it will be important to explain the impact of having an AMD SNP in terms that are relevant to the patient. It is generally accepted that the most relevant issue to the patient is not only visual function or the capacity of the visual system. In addition, patients are concerned with their functional vision or how well they can utilize their remaining vision in order to carry on their daily activities.

The SNPs with the largest genetic contribution to AMD are CFHY402H (Edwards et al., 2005; 2015; Kortvely et al., 2010) and ARMS2A69S (Ersoy et al., 2015; Kortvely et al., 2010; Schaumberg, Hankinson, Guo, Rimm, & Hunter, 2007). In the Quebec population, from which this study has sampled, the AMD SNP rs174547 in FADS1 appears to have an unusually high prevalence. Due to those factors, the latter three SNPs were selected for inclusion in the present study investigating their relationship to visual function. It was expected that carriers of these SNPs would experience greater functional losses compared to non-carriers, with homozygotes displaying the greatest functional impairment overall.

Methods

The same individuals who were tested in the first segment of this research also participated in this part of the study. The results of their genetic tests were taken into account herein.

Visual function was evaluated using a variety assessment tools. These included a general questionnaire of demographics and visual history, distance visual acuity, contrast sensitivity, colour vision, retinal sensitivity, location of fixation and fixation stability.

Visual acuity was measured using the EDTRS chart (See Appendix B). This chart was created to eliminate the design flaws of earlier visual acuity charts. The chart is more accurate due to the incorporation of specific criteria, including the same number of letters per row, equal spacing of rows, equal spacing of letters in a row and individual rows being balanced for letter difficulty. To prevent memorization, different charts were used for assessing left-eye, right-eye and binocular acuity.

Contrast sensitivity was measured using the Mars test (See Appendix B). With this test, it is the contrast, and not the letter (or numeral) size, which diminishes from the beginning to the end of the chart, thus controlling for visual acuity.

Colour vision was assessed using the Farnsworth Dichromatous-15 (D-15) Colour Arrangement Test (See Appendix B). The task requires arrangement of a set of coloured discs in a series. Those with colour deficits are known to make errors in sequencing the discs. Based on these mistakes, the type of colour deficiency can be determined.

Fixation stability was measured using the Scanning Laser Ophthalmoscope function of the Optos OCT/SLO (Figure 2). The SLO component uses confocal scanning laser microscopy to view the retinal surface in real time. The operator is able to view a participant's retina as he/she looks at a projected image. This allows identification of the location of fixation on the retina and its stability.

The fixation stability task requires the participant to gaze as steadily as possible at a target for a period of 20-seconds. An automatic eye tracker compensates for eye movements during the test period. The final output of this test is the superimposition of all the photos taken during the 20-second time frame. In the case of stable fixation, the target crosses are clustered together on the retina while unstable fixation would display the crosses spread out. Fixation stability was quantified using a bivariate contour ellipse area (BCEA). This is the area into which 95% of the target crosses fall, measured in square degrees. A smaller area is indicative of better fixation stability.

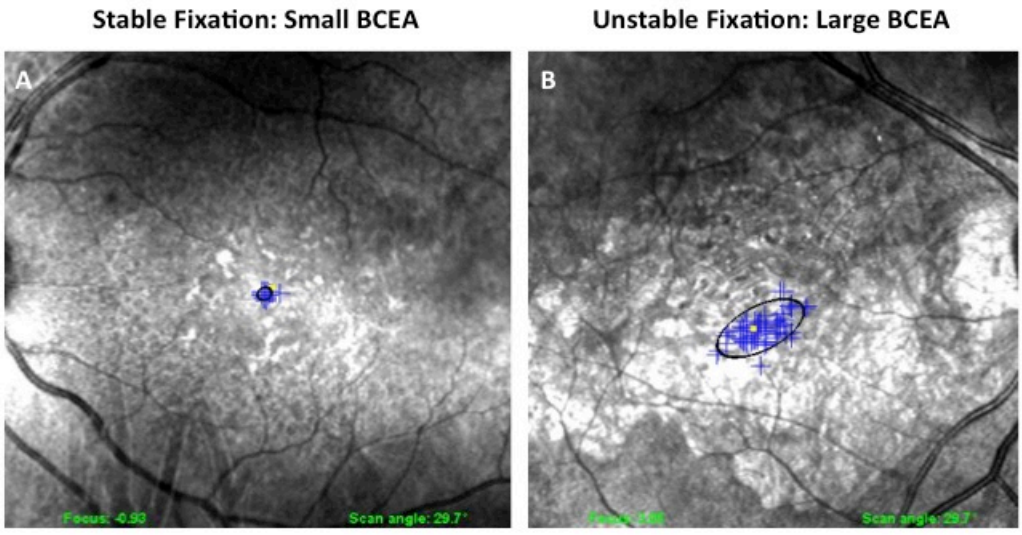

Figure 2. Bivariate Contour Ellipse Area (BCEA).

The BCEA is used to quantify fixation stability. An ellipse is drawn around the centre of fixation, with 95% of the fixation points within the ellipse. The area, measured in square degrees represents fixation stability. Typically, a BCEA less than 2 square degrees is considered stable fixation, while a BCEA greater than 2 square degrees is considered unstable fixation (Schuchard, 2005).

The location of fixation or preferred retinal locus (PRL) is determined using the results of the fixation stability test with the retinal topography function of the OCT/SLO. Retinal topography measures the volume of the retinal layers. Such a scan allows the identification of the anatomical fovea. Once the fovea is located, the image can be superimposed over the result of the fixation test and the distance between anatomical fovea and PRL can be measured.

Analysis

All calculations were performed using SPSS software, version 20.0 (IBM Corp, 2011; JASP Team, 2017). Chi Square was used to compare the results of the Farnsworth D-15 between AMD and controls and between carriers and non-carriers of each SNP. Independent Student's t tests were used to compare the means of visual acuity, contrast sensitivity, BCEA and eccentricity between AMD and controls and between carriers and non-carriers of each SNP of interest. Since the data did not follow a normal distribution, the nonparametric Mann-Whitney U test was also used to compare mean ranks. One-way ANOVA was used to compare the results of visual function against the zygosity of each SNP of interest.

Results

As expected, the AMD group performed significantly worse on traditional measures of visual function. Figures 3 and 4 present the results for visual acuity and contrast sensitivity. The Farnsworth D-15 (Figure 5) showed that individuals with AMD were more likely to have difficulty with colour vision than controls, χ^2=9.079, p=0.003, particularly in the tritan range of the spectrum, χ^2=11.17, p=0.011. Those with AMD also had larger BCEA values. A large BCEA value is interpreted as poor fixation stability, indicating that individuals with AMD have poorer fixation stability compared to controls, but the difference was not statistically significant (Figure 6). The AMD group also had greater eccentricity of fixation when compared to the control group, however this difference was also not statistically significant either (Figure 7).

	Test	Statistic	df	p	d	95% CI	
						Lower	Upper
LogMAR VA OU	Student	4.026	37	<0.001		0.144	0.436
	MWU	323.5		<0.001	1.293	0.120	0.370

Descriptive Statistics

	Group	N	Mean	SD	SE
LogMAR VA OU	AMD	21	0.273	0.288	0.063
	Control	18	-0.017	0.108	0.025

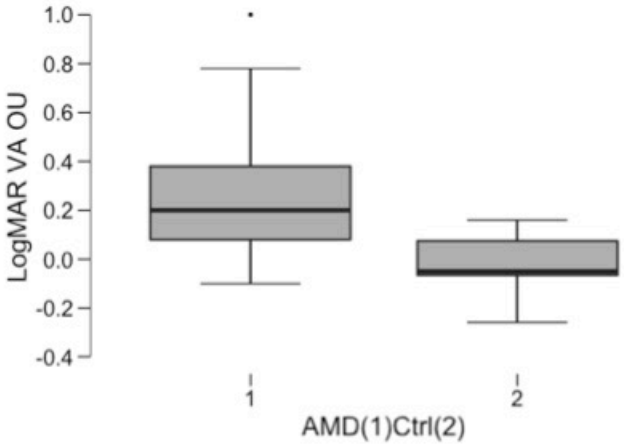

Figure 3. Binocular Visual Acuity in AMD Group Versus Control Group

The graph shows the AMD group (1) and control group (2) on the x-axis plotted against logMAR binocular visual acuity on the y-axis. An independent Student's t test shows a significant difference between mean logMAR visual acuity in the AMD group versus the control group. The Mann-Whitney U test compared mean ranks and showed the same result.

	Test	Statistic	df	p	d	95% CI	
						Lower	Upper
Mars OU	Student	-4.475	37.00	<0.001	-1.437	-0.487	-0.183
	MWU	62.00		<0.001	-1.437	-0.480	-0.160

Descriptive Statistics

	Group	N	Mean	SD	SE
Mars OU	AMD	21	1.300	0.268	0.058
	Control	18	1.636	0.184	0.043

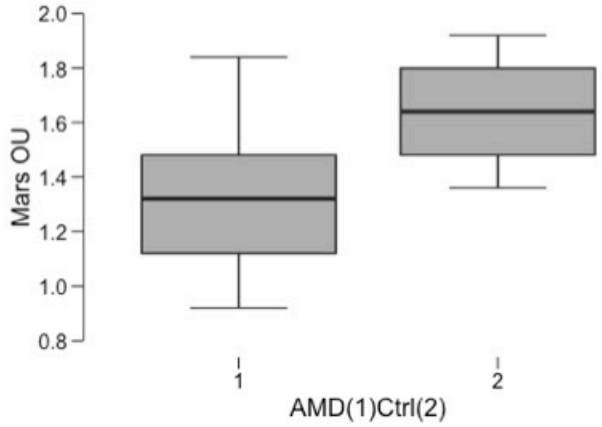

Figure 4. Mars Contrast Sensitivity in AMD Group versus Control Group

An independent Student's t test showed a significant difference between the mean contrast sensitivity of the AMD group and control group. The Mann-Whitney U test (MWU) supported this result. The boxplot shows the Mars contrast sensitivities in log units (y-axis) of the AMD group and the control group, 1 and 2 on the x-axis, respectively. Contrast sensitivity was measured binocularly.

A

Contingency Table: Farnsworth D15

Group	Pass	Fail	Total
AMD	7	13	20
Control	15	3	18
Total	16	22	38

Chi-Squared Test

	Value	df	p
χ^2	9.079	1	0.003
N	38		

B

Contingency Table: Farnsworth D15

	Normal	Protan	Tritan	Unknown	Total
AMD	7	1	11	1	20
Control	17	1	1	1	18
Total	22	2	12	2	38

Chi-Squared Test

	Value	df	p
χ^2	11.17	3	0.011
N	38		

Figure 5. The Farnsworth D15.

(A) shows the contingency table and Chi-squared test for those that passed or failed from the AMD group and the control group. A greater number of the AMD group were unable to pass the colour test compared to the control group. A *p* value of 0.003 indicates this difference is statistically significant. (B) shows the Chi-squared test taking the different colour axes into account. Those that fall under 'Normal' successfully passed the D15 while those under 'Protan' or 'Tritan' made errors along that colour axis. The 'Unknown' category represents those that made errors that did not fall under a particular colour axis. There were no participants who qualified as 'Deutan'.

	Test	Statistic	df	p	Cohen's d	95% CI	
						Lower	Upper
Log BCEA	Student	1.281	36	0.209	0.416	-0.133	0.589
	MWU	229.5		0.152	0.416	-0.110	0.484

Descriptive Statistics

	Group	N	Mean	SD	SE
Log BCEA	AMD	20	-0.002	0.636	0.142
	Control	18	-0.230	0.430	0.101

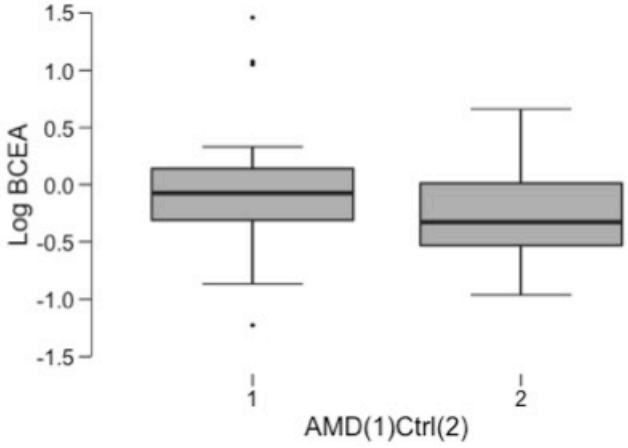

Figure 6. Fixation Stability of AMD Group versus Control Group.

BCEA was converted to a log10 scale to correct for skewness. An independent Student's t test showed no significant difference in mean log BCEA between the AMD group and controls. The Mann-Whitney U test (MWU) supported this result. The boxplot shows the AMD group (1) versus control group (2) on the x-axis against log BCEA on the y-axis.

	Test	Statistic	df	p	Cohen's d	95% CI	
						Lower	Upper
Log Ecc	Student	1.775	36	0.084	0.577	-0.038	0.564
	MWU	222.5		0.219	0.577	-0.108	0.433

Descriptive Statistics

	Group	N	Mean	SD	SE
Log Ecc	AMD	20	0.027	0.528	0.118
	Control	18	-0.236	0.359	0.085

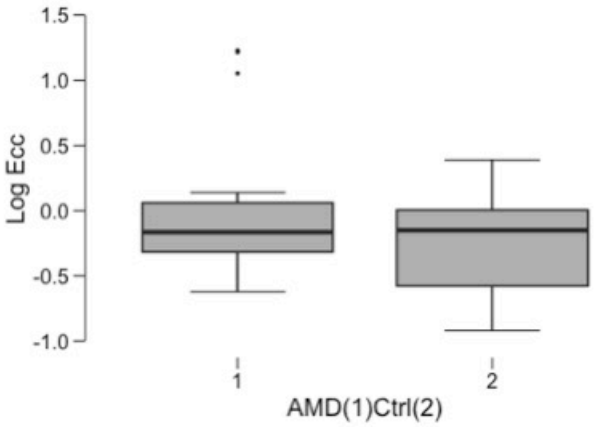

Figure 7. Eccentricity of Fixation.

The eccentricity of fixation, or PRL, is measured in degrees from the anatomical fovea, which is considered zero, to the centre of fixation (BCEA). Eccentricity was converted to a log10 scale to correct for skewness. The AMD group has a greater average eccentricity compared to the control. Means are not significantly different according to an independent Student's t test. This is supported by the Mann-Whitney U test comparing mean ranks. The boxplot shows the AMD group (1) and the control group (2) on the x-axis against log eccentricity on the y-axis.

Carriers of the CFHY402H appear to fixate with greater eccentricity than those who do not carry the SNP, but statistical significance is only achieved when zygosity is considered, $F(2,34)=9.53$, $p<0.001$ (Figure 8). This greater eccentricity does not appear to affect visual acuity or fixation stability. There was no significant difference between carriers and non-carriers in terms of contrast sensitivity or colour vision.

Homozygous carriers of ARMS2A69S showed poorer visual acuity and contrast sensitivity compared to heterozygotes and non-carriers (Figures 9 & 10). There were no statistically significant differences between carriers and non-carriers for colour vision, fixation stability or eccentricity. There were no significant trends with respect to visual function and the FADS1 SNP.

The demographic questionnaire collected data about lifestyle that has been known to influence the development of AMD. Smoking history was recorded in terms of number of years and number of cigarettes smoked per day. These parameters were used to calculate 'smoking dose' which is a measure of the number of cigarettes smoked per year spent smoking. The number of years spent smoking and smoking dose but not the number of cigarettes per day correlated with binocular visual acuity (Figure 11), BCEA (Figure 12) and eccentricity (Figure 13), even when age was controlled for. Sun protection was recorded in terms of how often the participant wore sunglasses. One-way ANCOVA controlling for age showed those who reported never wearing sunglasses had an earlier age of AMD onset compared to those who did wear sunglasses $F(3,29) = 3.589$, $p = 0.025$ (Figure 14).

Discussion

Smoking has been established as a risk factor for AMD by several studies (C Delcourt et al., 1998; W. Smith et al., 1996; Vingerling et al., 1996). This study agrees with a meta-analysis published in 2005, in that there is a relationship between the number of years spent smoking and severity of AMD (Thornton et al., 2005). The current study

ANOVA

	Sum of Squares	df	Mean Square	F	p
CFHY402H	778.8	2	389.39	9.745	<0.001

Post Hoc - Bonferroni

Zygosity		Mean Difference	SE	t	p
0	1	-1.124	2.349	-0.479	1.000
	2	-12.225	2.893	-4.225	<0.001
1	2	-11.101	2.926	-3.794	0.002

Descriptive Statistics

Zygosity	Mean (degrees)	SD	N
0	0.695	0.507	15
1	1.819	1.403	14
2	12.920	14.660	7

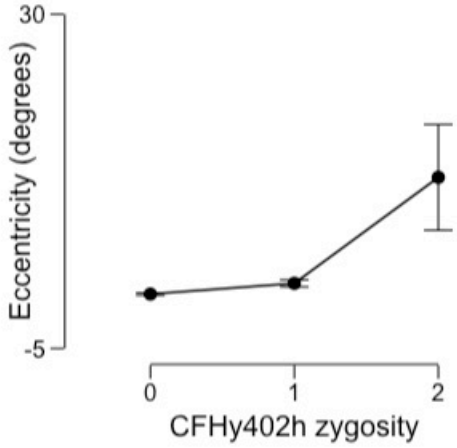

Figure 8. CFHY402H Carriers and Eccentricity of Fixation.

One-way ANOVA showed a highly significant difference between CFHY402H zygosity and eccentricity of fixation. The graph shows zygosity along the x-axis with '0' representing non-carriers, '1' representing heterozygotes, and '2' representing homozygotes and eccentricity from fovea on the y-axis (in degrees). Post-hoc analysis showed that homozygotes fixated the most eccentrically.

ANOVA

	Sum of Squares	df	Mean Square	F	p
ARMS2A69S	6.292	2	3.146	14.30	<0.001

Post Hoc - Bonferroni

Zygosity		Mean Difference	SE	t	p
0	1	0.095	0.164	0.581	1.000
	2	-1.063	0.221	-4.810	<0.001
1	2	-1.159	0.227	-5.115	<0.001

Descriptive Statistics

Zygosity	Mean (logMAR VA)	SD	N
0	0.377	0.383	18
1	0.281	0.331	15
2	1.440	0.883	6

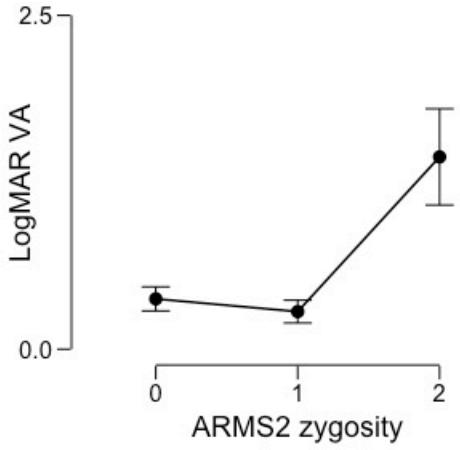

Figure 9. ARMS2A69S Carriers and Visual Acuity

One-way ANOVA showed a highly significant difference between the logMAR visual acuities depending of the zygosity of the ARMS2 SNP. The graph shows the zygosity along the x-axis with '0' representing non-carriers, '1' representing heterozygotes, and '2' representing homozygotes and logMAR visual acuity on the y-axis. A higher logMAR value indicates poorer visual acuity. Post-hoc analysis showed that homozygote carriers had poorer visual acuity than heterozygotes or non-carriers.

ANOVA

	Sum of Squares	df	Mean Square	F	p
ARMS2A69S	1.527	2	0.764	4.665	0.016

Post Hoc - Bonferroni

Zygosity		Mean Difference	SE	t	p
0	1	-0.053	0.141	-0.377	1.000
	2	0.520	0.191	2.727	0.029
1	2	0.573	0.195	2.934	0.017

Descriptive Statistics

Zygosity	Mean (Contrast)	SD	N
0	1.253	0.420	18
1	1.307	0.300	15
2	0.733	0.570	6

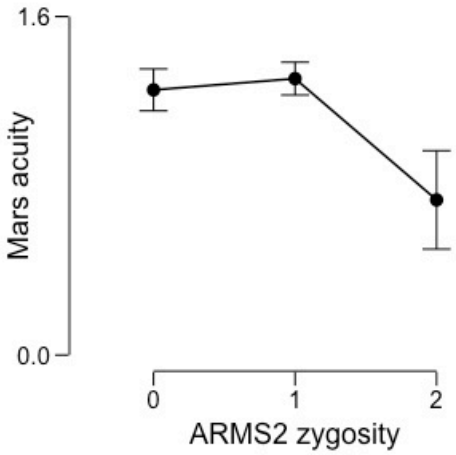

Figure 10. ARMS2A69S Carriers and Mars Contrast Sensitivity.

One-way ANOVA showed a significant difference in contrast sensitivity with respect to ARMS2A69S SNP zygosity. Post-hoc analysis showed homozygous carriers to have poorer contrast sensitivity compared to heterozygotes or non-carriers. The graph shows ARMS2 zygosity along the x-axis with '0' representing non-carriers, '1' representing heterozygotes, and '2' representing homozygotes. The y-axis displays the log score of Mars contrast sensitivity. A higher score indicates better contrast sensitivity.

went a step further, finding a correlation between years smoking and visual function in terms of visual acuity, fixation stability and eccentricity.

It was assumed that the AMD group would perform worse than the control group in terms of visual function. This was true for all parameters except for eccentricity of fixation. Eccentricity is not a traditional means of measuring visual function, likely because SLO technology is not readily available in most clinical settings. Given that AMD affects the central retina, it would make sense for those with the disease to be fixating more eccentrically compared to individuals with a healthy fovea. However, this is not always the case. These results agree with earlier reports from Reinhard *et al*. in 2007 and Greenstein *et al*. in 2008. Scotomas come in different shapes and sizes and it is possible for the fovea to be spared (Schuchard, 2005; Sunness, Rubin, Zuckerbrod, & Applegate, 2008), resulting in the lack of difference in average eccentricity between the AMD group and controls.

The Farnsworth D-15 showed that the AMD group experienced more difficulty distinguishing colour compared to the control group. Those with AMD had the most difficulty in the tritan spectrum, which encompasses blue-yellow wavelengths of light. The ability to distinguish different colours in this spectrum is known to decrease with age (Schneck, Haegerstrom-Portnoy, Lott, & Brabyn, 2014; Werner, Bayer, Schwarz, Zrenner, & Paulus, 2010). It appears that individuals with AMD have an exaggerated age-related effect. This is likely due to a combination of blue wavelength cones being the fewest number in the retina and most susceptible to damage by bright visible light (Cruickshanks, Klein, & Klein, 1993; Tomany, Cruickshanks KJ, Klein R, Klein BE, 2004). Further study with the use of an anomalscope could be used to quantify this effect.

Individuals carrying the CFHY402H SNP had significantly greater eccentric fixation compared to non-carriers. Those with two copies of the mutation displayed greater eccentricity than either non-carriers or single-copy carriers. The CFH SNP has been linked to increased drusen deposition, especially within 500μm of the fovea (Chang et al., 2014; C Delcourt et al., 2011). Since drusen are not efficiently removed in AMD, they would create permanent damage to the fovea, forcing fixation outward. Additionally,

persons carrying two mutated copies of CFH have been shown to have more central drusen than single copy carriers or non-carriers (Chang et al., 2014). This reflects the results of eccentricity in this study.

It has been well documented that visual acuity decreases with increasing eccentricity (Provis, Dubis, Maddess, & Carroll, 2013). With this, it is expected that fixation stability would decrease as well, though research shows mixed results (Greenstein et al., 2008; Reinhard et al., 2007). The current study showed, when all subjects were pooled, that increasing eccentricity significantly correlated with poorer visual acuity and less stable fixation. When subjects were separated into carriers versus non-carriers of CFHY402H, carriers had significantly greater eccentricity, but showed no significant difference in visual acuity or fixation stability. Some research has explored the plasticity of the oculomotor system in central vision loss and the training of a preferred retinal locus. They have shown that fixation stability can be improved and subjects can experience gains in reading speed and letter acuity (Nilsson et al., 1998, 2003; Tarita-Nistor, Gonzalez, Mandelcorn, Lillakas, & Steinbach, 2009), giving support to the old adage "practice makes perfect". Perhaps that is what happened in the case of the CFHY402H carriers. CFHY402H contributes to the development of early AMD (Dietzel et al., 2014). It is likely that carriers have had eccentric fixation longer and have had more practice, reducing the expected discrepancy in visual acuity and fixation stability.

Carriers of the ARMS2A69S appear to have poorer visual function in more traditional terms (visual acuity and contrast sensitivity). The parameters of visual acuity and contrast sensitivity are dependent on the integrity of the photoreceptors themselves. Given ARMS2 expression is localized specifically to the photoreceptors (Gatta et al., 2008; Katta et al., 2009), it makes sense that a mutation in ARMS2 would lead to a deficit in photoreceptor function reflected by poorer visual acuity and contrast sensitivity.

Conclusion

The results of this study support what has become common knowledge; individuals with AMD have poorer visual function compared to age-matched controls

with normal vision. Interestingly, it appears the CFHY402H SNP may influence functional vision through structural damage to the central retina, leading to more eccentric fixation. Conversely, ARMS2A69S appears to play more of a role in the function of photoreceptors over contribution to structural damage. There were no significant differences on measures of visual function in terms of carriers versus non-carriers of the FADS1 T allele. If the FADS1 T allele has an effect on visual function, it is likely a subtle one that would require greater numbers of C allele homozygotes to be seen. Further research investigating these new hypotheses is required in order to definitively establish these links.

Chapter 4: Study 3 - Retinal Structure

Drusen are one of the primary diagnostic criteria in AMD and have been a topic of debate for over 150 years. They are small extracellular deposits of debris located under the retina and visible on fundus photos as yellow dots. In 1877, Meyer speculated that drusen began at Bruch's membrane and were due to excretions from the retinal pigment epithelium (RPE). He went on to state that they were not only associated with age, but also inflammation and the nutritional state of the retina (Loeffler & Lee, 1998; Meyer, 1877). Despite many research groups having studied drusen, attempting to determine their biogenesis and how to eliminate them, over a century later, clinicians and researchers are still lacking fundamental information and left searching for answers. The most important finding to date is that not all drusen are created equal. Unfortunately, the term 'drusen' is still used by clinicians, histologists and biochemists alike to describe the deposits in Bruch's membrane despite differing morphology and composition.

Most often, drusen are classified clinically through ophthalmoscopy, fundus photography and fundus angiography. Drusen can be referred to as hard or soft and size is taken into account when considering risk of progression (Bird et al., 1995). The hard type of drusen is found in the peripheral retina and occurs with increasing age. They are typically < 63μm in diameter, although they can be as large as 125μm if they are flat in appearance. The greater the number of hard drusen, the more likely one is to develop the soft type. Soft drusen are > 63μm and have more substance to them compared to hard drusen (Williams, Craig, Passmore, & Silvestri, 2009). It is speculated that drusen deposits contribute to photoreceptor cell dysfunction and death by obstructing the exchange of nutrients and debris between the choroid and RPE. Additionally, drusen attract inflammatory activity that triggers a cascade promoting apoptosis, cell death and choroidal neovascularization (Lotery & Trump, 2007; Luibl et al., 2006). Drusen can also be identified by pattern and retinal location - macular versus peripheral (Williams et al., 2009). One pattern that is often described is that of reticular pseudodrusen (RPD). They appear as interlacing yellow ribbons and are external to the RPE compared to other types, which are located within the RPE and Bruch's membrane. RPD are better observed via scanning laser ophthalmoscope (SLO), which is not commonly available clinically,

compared to fundus photography (Boddu et al., 2014). Other drusen patterns have been identified histologically (Anderson et al., 2004, 2002; Hageman et al., 2001) but have yet to be identified *in vivo*.

One instrument that has become widely available in clinical settings is the optical coherence tomographer (OCT). This technology has evolved such that the resolution and sampling rate allows visualization of drusen ultrastructure *in vivo*. In 2006, ultra-high resolution OCT was used to identify three distinct drusen patterns in dry AMD (Pieroni et al., 2006). Image resolution in this study was limited due to the nature of the time-domain scanning technique at the time. Since then, another study using spectral domain OCT was able to identify 17 different drusen patterns. These patterns resulted from the combination of subcategories under four different characteristics: drusen shape, internal reflectivity, homogeneity and presence or absence of overlying foci. The same study was the first to identify drusen with a core on OCT (Khanifar, Koreishi, Izatt, & Toth, 2008). It is thought the cores may correspond to vesicles composed of activated complement components in histological studies (Anderson et al., 2002; Hageman et al., 2001).

The association of the presence of drusen with AMD genotypes has been investigated over the last decade. The two most widely discussed mutations contributing to AMD are single nucleotide polymorphisms (SNPs), referred to as CFHY402H and ARMS2A69S (Edwards et al., 2005; Ersoy et al., 2015; Kortvely et al., 2010). SNPs are single base pair changes in a gene sequence. These particular SNPs lead to amino acid substitutions in the encoded proteins ultimately affecting protein function. One of these mutations is CFHY402H (Edwards et al., 2005; Haines et al., 2005; R J Klein et al., 2005; Patel et al., 2008). This SNP causes a histidine (H) to tyrosine (Y) substitution in the complement factor H (CFH) protein. The second SNP is ARMS2A69S, which is a serine (S) to alanine (A) substitution in the protein encoded by age-related maculopathy susceptibility gene 2 (Ding et al., 2009; Jakobsdottir et al., 2005).

Recent studies are in agreement that CFHY402H and ARMS2A69S contribute to the progression of AMD (Edwards et al., 2005; Ersoy et al., 2015; Kortvely et al., 2010; Yu, Reynolds, Rosner, Daly, & Seddon, 2012). Some report the CFH SNP as being

associated with early drusen formation (Boon et al., 2009; Dietzel et al., 2014; Yu et al., 2012) and the ARMS2 SNP as contributing to later drusen progression (Dietzel et al., 2014; Yu et al., 2012). Whether or not these SNPs are linked to the type of AMD, wet versus dry, is still unclear (Cheng et al., 2013; Chong et al., 2015; Droz et al., 2008). Others have investigated the presence of these SNPs with respect to the location and pattern of drusen deposition. Carriers of the ARMS2 SNP have been reported to have a higher incidence of RPD, described earlier. Carriers of the same SNP tend to display more drusen near choroidal vessels as well (Kortvely et al., 2010; Ueda-Arakawa et al., 2013). On the other hand, the CFH SNP has had mixed reports with some reporting an association with RPD (Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005; R J Klein et al., 2005) and others reporting a lower incidence of RPD (Boon et al., 2009; Chong et al., 2015; R T Smith et al., 2011; Ueda-Arakawa et al., 2013) The same SNP was associated with cuticular drusen, which appear as a series of small raised subretinal deposits not associated with a thickening of Bruch's membrane (Boon et al., 2009). Homozygous carriers of the CFH SNP were found to have greater numbers of central drusen, covering just over 50% of the central 500μm radial area of the macula with drusen (Chong et al., 2015). CFHY402H has been linked to peripheral drusen as well (Droz et al., 2008).

Few studies have attempted to link drusen to AMD genotypes through *in vivo* imaging, especially in the detail described by Khanifar *et al*. Drusen are still best characterized through post-mortem histological sections. A 2015 study was able to confirm a link between the CFH risk SNP and drusen area, volume, and RPE atrophy identified via OCT in an Amish population (Ramana et al., 2015). A link between AMD SNPs and the characterisitics of drusen visible on OCT would improve understanding of the pathogenesis of AMD and gain some of the fundamental information that is still lacking. This study aimed to investigate whether the most common AMD SNPs can be linked to drusen ultrastructure as seen on OCT.

Methods

Participants were recruited from the Montreal Retina Institute and the School of Optometry Clinic at the Université de Montréal. Study protocol was approved by *Le Comité d'éthique de la recherche en santé* at the university and followed the tenets of the Declaration of Helsinki. All study participants gave signed informed consent prior to their participation in the study.

Subjects aged 70 years or older and diagnosed with AMD by an ophthalmologist or optometrist we recruited for this study. Individuals with comorbid glaucoma, neurological disorders or a diagnosis of dementia were excluded.

For 10 participants from the patient group, genotyping was conducted as part of a previous study (Smailhodzic et al., 2012) by Radboud University Medical Center in Nijmegen, Netherlands. The remainder of the patient group and the control group were genotyped from saliva samples by Asper Biotech Ltd. in Estonia.

Retinal Structure

Retinal structure was evaluated using fundus photography and the OCT/SLO. Mydriadic eye drops were used to dilate pupils. At maximum dilation, colour fundus photos were taken using a Canon CR-1 fundus camera. Two optometrists evaluated fundus photographs using the Age-related Eye Disease Study (AREDS) grading schema (Davis et al., 2005). Fundus photos were also evaluated in terms of AMD type (wet or dry), pigment mottling, geographic atrophy, and drusen. Pigment mottling was assessed in terms of severity (none, mild, moderate or severe). Geographic atrophy was also assessed based on severity. The percentage of the macula affected by geographic atrophy was also considered. Drusen were categorized as small, medium or large based on the AREDS guidelines. The number of drusen in each size category was approximated into the following categories: zero, 1 to 5, 5 to 10, 10 to 25, 25 or more.

The Optos OCT/SLO raster scan function was used to take cross sectional images of the macular region in each eye. A total of 32 parallel cross-sectional scans are taken

from the top of the fundus to the bottom. A custom-made MATLAB program was used to identify drusen boundaries and characteristics based on four categories: shape, homogeneity, reflectivity and hyper-reflective foci (Table IV). Drusen were measured in terms of retinal area in square microns.

Analysis

All calculations were performed using SPSS software, version 20.0 (IBM Corp, 2011; JASP Team, 2017). Pearson's correlation was used to compare drusen characteristics identified via OCT to AMD characteristics identified via fundus photography. Kruskal-Wallis was used to compare SNP zygosity to the mean ranks of drusen characteristics. Due to the small sample size, an alpha < 0.1 was considered a significant result in statistical tests.

Results

A total of 19 eyes had OCT images viable for labelling. The average area covered by drusen per eye was 29,050 square microns in the better eye. Table V shows the descriptive statistics per drusen characteristic subcategory. In terms of shape, most fell under the concave subcategory (82.2%) followed by convex (13.3%) and then pointy (4.47%). Most drusen had a nonhomogeneous nature, with 18.86% having a core. Just over half of the drusenoid area was labelled as having high internal reflectivity (54.97%), followed by mid-reflectivity (28.41%) and low reflectivity (16.61%). Only a small percentage of the drusenoid area was identified as having hyper-reflective foci above the druse (13.09%).

Only 16 eyes had fundus photos clear enough for grading. The frequency distribution per AMD grade can be seen in Figure 11. AMD grade determined from fundus photography positively correlated with drusen area, drusen of concave and pointy shape, drusen of mid and high reflectivity, drusen with nonhomogeneous content, drusen with core and drusen with overlying hyperreflective foci from OCT scans. These correlations are displayed in Tables VI-VIII. Severity of geographic atrophy was positively

Table IV. Description of Drusen Characteristics

Characteristic	Description
Shape	The contour of the druse
Convex	Smooth dome-shaped elevation of RPE
Concave	Elevation of RPE with irregular shape
Pointy	Small, sharp elevation of RPE
Homogeneity	The appearance of the interior of the druse
Homogeneous	Uniform reflectivity within druse
Nonhomogeneous	Varying internal reflectivity
Core	Varying reflectivity with defined foci
Reflectivity	The brightness of the druse with respect to the RPE
High	Hyperreflective compared to the RPE
Medium	Isoreflective with the RPE
Low	Hyporreflective compared to the RPE
Hyperrreflective foci	The presence/absence of hyperreflective areas within the neurosensory retina above a druse

RPE: retinal pigment epithelium

Table V. Descriptive Statistics for Each Drusen Characteristic and Subcategory

Characteristic		Descriptive Statistics					
		Mean	SD	SE	Min	Max	%*
Shape	Convex	3863.0	9660.0	2216.0	0	41790.0	13.30
	Concave	23880.0	21870.0	5018.0	100.0	84080.0	82.20
	Pointy	1298.0	2211.0	507.2	0	9018.0	4.47
Homogeneous	Yes	479.4	849.9	195.0	0	2547.0	1.65
	No	23090.0	21570.0	4948.0	0	82230.0	79.48
	Core	5479.0	6384.0	1465.0	0	20430.0	18.86
Reflectivity	High	15970.0	15490.0	3553.0	100.0	5720.0	54.97
	Mid	8252.0	7795.0	1788.0	0	25750.0	28.41
	Low	4825.0	9345.0	2144.0	0	40670.0	16.61
Foci	Present	3804.0	4830	1108	0	14120.0	13.09

**Percentage of average drusenoid area*

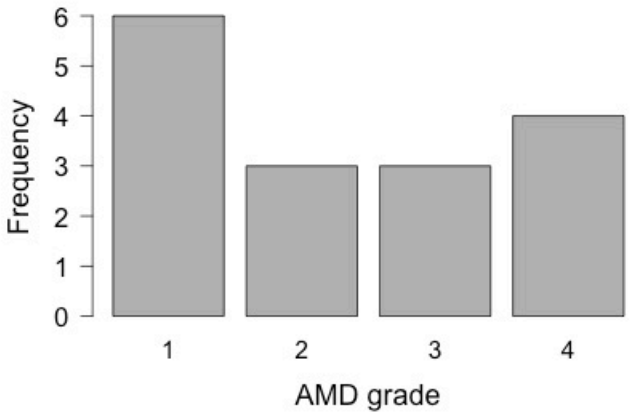

Figure 11. AMD Grade of Participants.

A total of 16 eyes had gradable fundus photos. This graph shows the distribution fundus photos falling under each AREDS category. The most participants fall into category 1, early AMD. The second most populated category is category 4, late AMD, which can be geographic atrophy or neovascularization.

Table VI. Correlation of AMD Grade with Drusen Area and Shape

		AMD Grade	Drusen Area	Convex	Concave	Pointy
AMD Grade	*r*	1	0.726**	0.061	0.741**	0.561**
	p		0.003	0.836	0.002	0.037
	N	16	14	14	14	14
Drusen Area	*r*		1	0.415	0.920***	0.376
	p			0.077	0.000	0.113
	N		19	19	19	19
Convex	*r*			1	0.035	-0.27
	p				0.887	0.913
	N			19	19	19
Concave	*r*				1	0.339
	p					0.155
	N				19	19
Pointy	*r*					1
	p					
	N					19

**p < 0.05, ***p < 0.001

AMD grade showed significant positive correlations with drusen area, and drusen that were concave or pointy in shape. This means that eyes with more advanced forms of AMD have more drusen and were more likely to have drusen classified as concave or pointy in shape.

Table VII. Correlation of AMD Grade with Drusen Reflectivity and Foci

		AMD Grade	High Reflectivity	Mid Reflectivity	Low Reflectivity	Foci
AMD Grade	*r*	1	0.756**	0.691**	0.110	0.689**
	p		0.002	0.006	0.707	0.006
	N	16	14	14	14	14
High Reflectivity	*r*		1	0.737***	0.113	0.804***
	p			0.000	0.646	0.000
	N		19	19	19	19
Mid Reflectivity	*r*			1	0.171	0.815***
	p				0.485	0.000
	N			19	19	19
Low Reflectivity	*r*				1	0.041
	p					0.868
	N				19	19
Foci	*r*					1
	p					
	N					19

**p < 0.05, ***p < 0.001

AMD grade had significant positive correlations with drusen of high or mid reflectivity and the presence of overlying hyperreflective foci. This means eyes with more advanced forms of AMD were more likely to have greater numbers of high or mid reflective drusen and overlying hyperreflective foci compared to eyes in earlier stages of disease.

Table VIII. Correlation of AMD Grade with Drusen Content

		AMD Grade	Homogeneous	Nonhomogeneous	Core
AMD Grade	r	1	0.380	0.645**	0.635**
	p		0.180	0.013	0.015
	N	16	14	14	14
Homogeneous	r		1	0.368	0.412
	p			0.121	0.080
	N		19	19	19
Nonhomogeneous (No core)	r			1	0.358
	p				0.132
	N			19	19
Core	r				1
	p				
	N				19

**p < 0.05, ***p < 0.001

AMD grade had significant positive correlations with drusen of nonhomogeneous content, with and without cores. The more AMD had progressed in the eye, the more nonhomogeneous drusen it was likely to have.

correlated with drusen area, drusen concave in shape, drusen of mid and high reflectivity, drusen with nonhomogeneous content and drusen with overlying hyperreflective foci. These correlations are displayed in Tables IX-XI. The percentage of the macula already covered with geographic atrophy was positively correlated with drusen of mid reflectivity and drusen with core (Tables XII and XII). Drusen characteristics visible on OCT did not show any significant correlations with drusen size or severity of pigment mottling as determined from fundus photos.

When CFHY402H zygosity was correlated with drusen OCT characteristics, there were significant positive correlations with drusen area, $r = 0.411$, $p = 0.081$, and drusen of mid reflectivity, $r = 0.449$, $p = 0.054$ (Tables XIV-XVI). Kruskal-Wallis showed that homozygotes had a greater percentage of their macula affected by geographic atrophy compared to other participants, $H(2) = 6.603$, $p = 0.037$ (Figure 12).

ARMS2A69S zygosity also correlated with drusen of mid reflectivity ($r = 0.527$, $p = 0.020$), but to a slightly higher degree than CFHY402H. The ARMS2 SNP also showed a positive correlation with drusen of homogenous content ($r = 0.413$, $p = 0.079$). There were no significant correlations between the FADS1 SNP zygosity and drusen characteristics. The correlations of drusen characteristics and ARMS2A69S zygosity can be seen in Tables XVII-XX. Similarly, Kruskal-Wallis analysis did not show any significant trends between the ARMS2 or FADS1 SNPs and drusen characteristics.

Discussion

Drusen Characteristics and Genetics

Both CFHY402H and ARMS2A69S SNPs correlated with drusen of mid reflectivity. A large percentage of drusen were labelled as having mid reflectivity in this study, while it was the highest occurring pattern of reflectance reported in Khanifar *et al*. (2008). This leads to speculation that mid reflectivity is related to pathogenesis over low reflectivity, which was of low prevalence in the current study and in Khanifar *et al*, 2008.

Table IX. Correlation of GA Severity with Drusen Area and Shape

		GA	Drusen Area	Convex	Concave	Pointy
GA	*r*	1	0.621**	-0.019	0.681**	0.361
	p		0.018	0.947	0.007	0.205
	N	16	14	14	14	14
Drusen Area	*r*		1	0.415	0.920**	0.376
	p			0.077	0.000	0.113
	N		19	19	19	19
Convex	*r*			1	0.035	-0.27
	p				0.887	0.913
	N			19	19	19
Concave	*r*				1	0.339
	p					0.155
	N				19	19
Pointy	*r*					1
	p					
	N					19

**p < 0.05, ***p < 0.001

The severity of GA had significant positive correlations with drusen area and drusen that were classified as concave in shape. This means that eyes with more severe GA, had more retina covered by drusen and more of those drusen were classified as concave compared to eyes with less severe GA.

Table X. Correlation of GA Severity with Drusen Reflectivity and Foci

		GA	High Reflectivity	Mid Reflectivity	Low Reflectivity	Foci
GA	*r*	1	0.657**	0.702**	-0.003	0.573**
	p		0.011	0.005	0.991	0.032
	N	16	14	14	14	14
High Reflectivity	*r*		1	0.737***	0.113	0.804***
	p			0.000	0.646	0.000
	N		19	19	19	19
Mid Reflectivity	*r*			1	0.171	0.815***
	p				0.485	0.000
	N			19	19	19
Low Reflectivity	*r*				1	0.041
	p					0.868
	N				19	19
Foci	*r*					1
	p					
	N					19

**p < 0.05, ***p < 0.001

The severity of GA had significant positive correlations with drusen of high and mid reflectivity and overlying hyper-reflective foci. Eyes with more severe GA had drusen of greater reflectivity and more overlying foci compared to eyes with less severe GA.

Table XI. Correlation of GA Severity with Drusen Content

		GA	Homogeneous	Nonhomogeneous	Core
GA	r	1	0.338	0.577**	0.438
	p		0.237	0.031	0.117
	N	16	14	14	14
Homogeneous	r		1	0.368	0.412
	p			0.121	0.080
	N		19	19	19
Nonhomogeneous (No core)	r			1	0.358
	p				0.132
	N			19	19
Core	r				1
	p				
	N				19

**p < 0.05, ***p < 0.001

GA severity had a significant, positive correlation with drusen of nonhomogeneous content.

Table XII. Correlation of Percentage of the Macula Affected by GA with Drusen Area and Shape

		GA%	Drusen Area	Convex	Concave	Pointy
GA%	*r*	1	0.408	0.033	0.419	0.297
	p		0.148	0.910	0.136	0.303
	N	16	14	14	14	14
Drusen Area	*r*		1	0.415	0.920***	0.376
	p			0.077	0.000	0.113
	N		19	19	19	19
Convex	*r*			1	0.035	-0.27
	p				0.887	0.913
	N			19	19	19
Concave	*r*				1	0.339
	p					0.155
	N				19	19
Pointy	*r*					1
	p					
	N					19

**p < 0.05, ***p < 0.001

The percentage of the macula affected by GA was not significantly correlated with drusen area or drusen shape, even when $p < 0.1$ was considered significant.

Table XIII. Correlation with Percentage of Macula Affected by GA with Drusen Reflectivity and Foci

		GA%	High Reflectivity	Mid Reflectivity	Low Reflectivity	Foci
GA%	*r*	1	0.390	0.519	0.018	0.358
	p		0.168	0.057	0.951	0.209
	N	16	14	14	14	14
High Reflectivity	*r*		1	0.737***	0.113	0.804***
	p			0.000	0.646	0.000
	N		19	19	19	19
Mid Reflectivity	*r*			1	0.171	0.815***
	p				0.485	0.000
	N			19	19	19
Low Reflectivity	*r*				1	0.041
	p					0.868
	N				19	19
Foci	*r*					1
	p					
	N					19

**p < 0.05, ***p < 0.001

If $p < 0.1$ is considered significant, then the percentage of the macula affected by drusen showed a significant, positive correlation with drusen of mid reflectivity.

Table XIV. Correlation of Percentage of Macula Affected by GA with Drusen Content

		GA%	Homogeneous	Nonhomogeneous	Core
GA%	r	1	0.3305	0.319	0.517
	p		0.290	0.266	0.058
	N	16	14	14	14
Homogeneous	r		1	0.368	0.412
	p			0.121	0.080
	N		19	19	19
Nonhomogeneous (No core)	r			1	0.358
	p				0.132
	N			19	19
Core	r				1
	p				
	N				19

**p < 0.05, ***p < 0.001

If $p < 0.1$ is considered significant, then the percentage of the macula affected by drusen showed a significant, positive correlation with nonhomogeneous drusen possessing a core.

Table XV. Correlations of CFHY402H Zygosity with Drusen Area and Shape

		CFHY402H	Drusen Area	Convex	Concave	Pointy
CFHY40H Zygosity	*r*	1	0.411*	0.162	0.375	0.214
	p		0.081	0.508	0.113	0.378
	N	21	19	19	19	19
Drusen Area	*r*		1	0.415	0.920**	0.376
	p			0.077	0.000	0.113
	N		19	19	19	19
Convex	*r*			1	0.035	-0.27
	p				0.887	0.913
	N			19	19	19
Concave	*r*				1	0.339
	p					0.155
	N				19	19
Pointy	*r*					1
	p					
	N					19

*$p < 0.10$, **$p < 0.05$, ***$p < 0.001$

When $p < 0.10$ was considered significant, Pearson's correlation showed a significant, positive trend between CFH SNP zygostiy and drusen area. Individuals with more copies of CFHY402H had greater areas of drusen in their better eye.

Table XVI. Correlations of CFHY402H Zygosity with Drusen Reflectivity and Foci

		CFHY402H Zygosity	High Reflectivity	Mid Reflectivity	Low Reflectivity	Foci
CFHY402H Zygosity	*r*	1	0.330	0.449*	0.176	0.317
	p		0.167	0.054	0.472	0.186
	N	21	19	19	19	19
High Reflectivity	*r*		1	0.737***	0.113	0.804***
	p			0.000	0.646	0.000
	N		19	19	19	19
Mid Reflectivity	*r*			1	0.171	0.815***
	p				0.485	0.000
	N			19	19	19
Low Reflectivity	*r*				1	0.041
	p					0.868
	N				19	19
Foci	*r*					1
	p					
	N					19

*$p < 0.10$, **$p < 0.05$, ***$p < 0.001$

When $p < 0.10$ was considered significant, Pearson's correlation showed a significant positive trend with respect to CFH SNP zygosity and drusen of mid reflectivity. Individuals with more copies of CFHY402H had greater areas of drusen with mid reflectivity.

Table XVII. Correlations of CFHY402H Zygosity with Drusen Content

		CFHY402H	Homogeneous	Nonhomogeneous	Core
CFHY402H Zygosity	r	1	0.087	0.379	0.313
	p		0.724	0.109	0.192
	N	21	19	19	19
Homogeneous	r		1	0.368	0.412
	p			0.121	0.080
	N		19	19	19
Nonhomogeneous (No core)	r			1	0.358
	p				0.132
	N			19	19
Core	r				1
	p				
	N				19

*$p < 0.10$, **$p < 0.05$, ***$p < 0.001$

There were no significant correlations between drusen content and CFHY402H zygosity, even when $p < 0.10$ was considered significant.

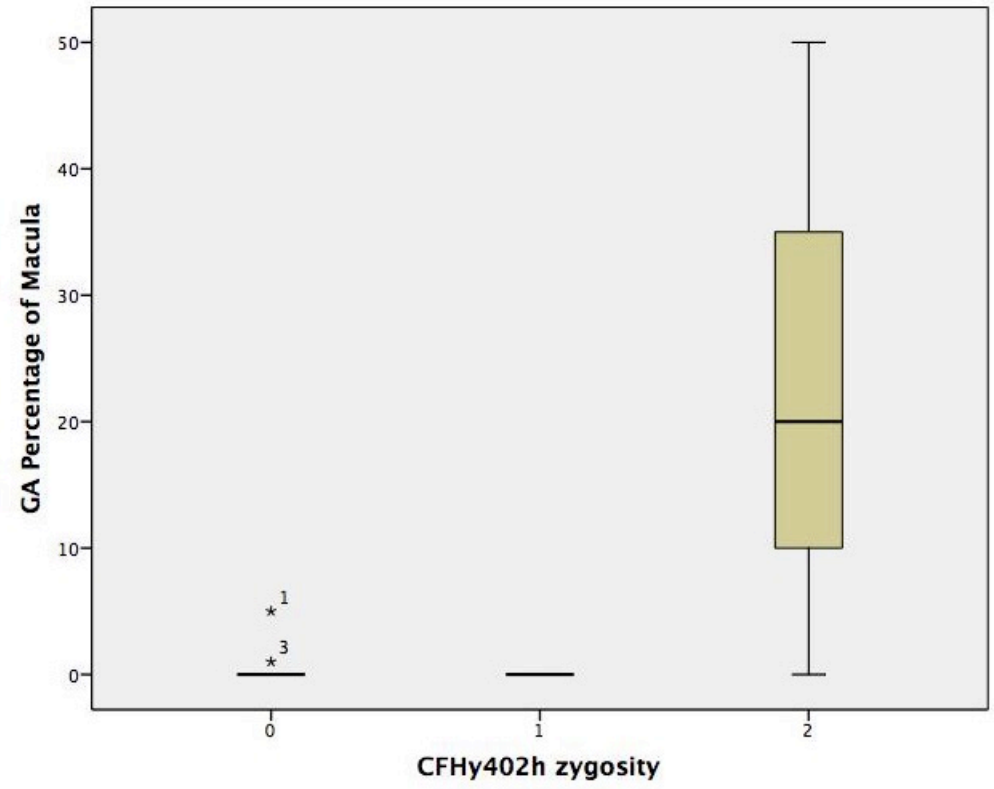

Figure 12. Percentage of the Macula Affected by GA According to CFHY402H Zygosity. This boxplot displays the number of copies of the CFHY402H SNP carried (x-axis) versus the percentage of the macula affected by GA (y-axis). Kruskal-Wallis showed a significant difference between mean ranks of affected macular area according to CFH SNP zygosity, $H(2) = 6.603$, $p = 0.037$. Individuals with AMD having two copies of the SNP had a greater average percentage of macula affected by GA in the better eye compared to those carrying one or no copies of the SNP.

Table XVIII. Correlation of ARMS2A69S with Drusen Area and Shape.

		ARMS2A69S	Drusen Area	Convex	Concave	Pointy
ARMS2A69S Zygosity	*r*	1	0.356	0.200	0.346	-0.270
	p		0.134	0.411	0.147	0.263
	N	21	19	19	19	19
Drusen Area	*r*		1	0.415	0.920**	0.376
	p			0.077	0.000	0.113
	N		19	19	19	19
Convex	*r*			1	0.035	-0.27
	p				0.887	0.913
	N			19	19	19
Concave	*r*				1	0.339
	p					0.155
	N				19	19
Pointy	*r*					1
	p					
	N					19

*$p < 0.10$, **$p < 0.05$, ***$p < 0.001$

There were no significant correlations between ARMS2A69S zygosity and drusen area or shape, even when $p < 0.10$ was considered significant.

Table XIX. Correlation of ARMS2A69S Zygosity with Drusen Reflectivity and Foci.

		ARMS2A69S Zygosity	High Reflectivity	Mid Reflectivity	Low Reflectivity	Foci
ARMS2A69S Zygosity	*r*	1	0.178	0.527**	0.217	0.379
	p		0.465	0.020	0.372	0.109
	N	21	19	19	19	19
High Reflectivity	*r*		1	0.737***	0.113	0.804***
	p			0.000	0.646	0.000
	N		19	19	19	19
Mid Reflectivity	*r*			1	0.171	0.815***
	p				0.485	0.000
	N			19	19	19
Low Reflectivity	*r*				1	0.041
	p					0.868
	N				19	19
Foci	*r*					1
	p					
	N					19

*$p < 0.10$, **$p < 0.05$, ***$p < 0.001$

Pearson's correlation showed a significant, positive trend between ARMS2 SNP zygosity and drusen of mid reflectivity. Individuals with more copies of ARMS2A69S had more drusen of mid reflectivity.

Table XX. Correlations of ARMS2A69S Zygosity and Drusen Content.

		ARMS2A69S	Homogeneous	Nonhomogeneous	Core
ARMS2A69S Zygosity	r	1	0.413*	0.321	0.255
	p		0.079	0.181	0.292
	N	21	19	19	19
Homogeneous	r		1	0.368	0.412
	p			0.121	0.080
	N		19	19	19
Nonhomogeneous (No core)	r			1	0.358
	p				0.132
	N			19	19
Core	r				1
	p				
	N				19

*$p < 0.10$, **$p < 0.05$, ***$p < 0.001$

When $p < 0.10$ was considered significant, Pearson's correlation showed a significant, positive trend between ARMS2 SNP zygosity and homogeneous drusen. Individuals with more copies of ARMS2A69S had more drusen with homogeneous content.

The ARMS2 SNP was linked to drusen having homogeneous content. Although current literature does not speculate as to what the homogeneous content could represent, the correlation with ARMS2 suggests it may be drusen consisting mainly of photoreceptor material. The SNP could lead to the improper processing and/or turnover of photoreceptor components leading to their accumulation in the retina. In order for the accumulation to appear homogeneous, it would need to have little else accumulate with it, suggesting a homogeneous druse would appear before inflammatory proteins got involved. This would explain the low occurrence of homogeneous drusen if they all progress to become nonhomogeneous, but this theory does not agree with studies suggesting that the involvement of ARMS2A69S occurs in later drusen progression (Dietzel et al., 2014; Yu et al., 2012). There is still much speculation concerning ARMS2 and its actual function. More study of the wild type ARMS2 gene and histological study of homogeneous drusen would provide more evidence to support a better hypothesis concerning this correlation.

It was hypothesized that the presence of CFHY402H would be linked to the presence of drusen with core due to the similar structures being identified in histological studies. The zygosity of this SNP did not significantly correlate with the presence of drusen with core in this sample. This is likely due to the small sample size. An alternative theory could be that misfolded complement proteins that occur in drusen do not always form vesicular structures resembling the cores seen on OCT. Perhaps, another protein, like beta-amyloid, is required for misfolded complement proteins to interact with before they can form such an organized structure. If this were the case, it would be more likely to see a correlation between the other protein and drusen with core, and CFHY402H zygosity and drusen area. This alternative theory is supported by a significant, positive correlation between drusen area and CFHY402H zygosity when $p < 0.10$ was considered significant, $r = 0.411$, $p = 0.081$. A larger sample size would likely give this result more statistical power. Beta-amyloid was not a parameter that was measured in this study, but it has been shown to be present in the vesicular structures along with complement components (D H Anderson et al., 2004, 2002; Hageman et al., 2001). Currently, methods to identify beta-amyloid *in vivo* are still in their infancy. Recent research on the

polarization properties of the protein (Campbell et al., 2015; Hamel et al., 2016) show *in vivo* identification may be possible via two-photon microscopy in the not too distant future (Avila et al., 2015). Even more recently, the natural fluorochrome curcumin, was formulated to be used as a probe to identify beta-amyloid *in vivo* through a modified SLO (Koronyo et al., 2017).

In order to establish if any relationship between the FADS1 SNP and retina structure exist, a greater proportion of non-carriers would be required in the sample. This would likely be achieved with recruitment outside Quebec given the potential of a FADS1 Founder Effect. The current sample has only three participants with AMD and without the mutation. The FADS1 SNP could potentially contribute to retinal damage through several different mechanisms (described in Chapter 2), making it more likely to contribute to several different drusen characteristics.

Limitations

One of the major limitations of this study is sample size. There are a large number of drusen characteristics. Each characteristic must be well-defined and have enough eyes per subcategory to achieve proper statistical power. A larger proportion of participants with dry AMD would have also been helpful in better defining drusen characteristics. Dry AMD does not have the confounds of fluid and the scaring that occurs afterwards as is the case with wet AMD. Another limitation is intra-user and inter-user reliability in terms of labelling. Reliability is currently being established, but the labelling process is labour-intensive making it difficult to complete for most professionals. Recent research (Schlanitz et al., 2015; Schlanitz et al., 2010; Schlanitz et al., 2017) manually segmented drusen boundaries, but used an algorithm to label them, cutting down on labour. Sample size was also a limiting factor with respect to drusen characteristics. There are a large number of drusen patterns that could exist and a large sample size would be required to better define them all.

Future research will include the use of such algorithms, as well as machine learning to minimize labour and improve the accuracy of labelling. The study of drusen characteristics via OCT could provide more information than that available through

fundus photo grading. OCT images could provide a sub classification system for drusen provided characteristics can be correlated to data from biochemical studies based on cadaver eyes. Eventually, prospective studies on the characteristics may lead to models for prediction of progression to later stages.

Conclusion

The results of this study agree with previous literature showing a link between the CFH and ARMS2 risks SNPs, more severe forms of AMD, and drusen (Chong et al., 2015; Hoffman et al., 2016; Magnusson et al., 2006; Ueda-Arakawa et al., 2013). Both SNPs were related to drusen of mid reflectivity suggesting this characteristic may be indicative of disease progression. The CFHY402H SNP was not linked to the presence of a core within drusen as hypothesized, but this connection cannot be ruled out. CFHY402H was linked to drusen area and drusen of nonhomogeneous content, however an interaction between CFH and beta-amyloid may be a requirement for the formation of nonhomogeneous drusen with core. Recent research shows this may be possible to determine in the not too distant future (Avila et al., 2015; Koronyo et al., 2017).

Although this study provides some insight on the link between AMD risk genotype and retinal structure, it also highlights how much remains unknown about the origins of drusen and their progression. There are numerous studies on the genetics of AMD and the equivalent on the histopathology and imaging of drusen, but they largely remain separate domains. Only better communication among investigators in these domains will achieve increased understanding of the mechanism that begins with AMD risk SNPs and leads to the formation of drusen, the hallmark of the disease.

Chapter 5: Study 4 - Cognitive Function

With the aging of the Canadian population, the number of individuals affected by Age-related Macular Degeneration (AMD) is on the rise. AMD is presently the leading cause of legal blindness in industrialized nations with a prevalence that increases with age (R J Klein et al., 2007). This pathology impairs, among others, the ability to read, to recognize faces, and to drive, all of which can lead to a decreased quality of life and loss of autonomy. To date, AMD is understood to be a degenerative condition with few treatment options.

In addition to a higher prevalence of AMD, increased aging of the population will result in a higher prevalence of age-related cognitive impairment. At present, the World Health Organization estimates that approximately 5-7% of the population aged 60 and over suffers from cognitive impairment (World Health Organization, 2012b; Wortmann, 2012), with a large increase in the absolute number of individuals affected predicted with the shift in demographics. Cognitive impairment refers to a decrease in a person's ability to remember and think, to the extent that it interferes with the ability to perform daily activities.

Not only does the prevalence of both AMD and cognitive impairment increase with age, there is a growing body of scientific literature linking the two. This started around the turn of the millennium with large-scale population-based studies reporting a higher prevalence of cognitive impairment among individuals with AMD (Klaver et al., 1999; Wong et al., 2002). In the first decade of the 2000s, research determining shared risk factors and histopathological characteristics began to surface (Anderson et al., 2004; L V Johnson et al., 2002; Katta et al., 2009; Terai et al., 2001). Since then, with advances in science and technology and the data released from genome-wide association studies (GWAS), researchers have developed their abilities to study complex diseases. A complex disease is caused by a combination of genetic, environmental and lifestyle factors. Characterizing the contribution of a factor to a complex disease is difficult due to the factor being obscured or confounded by other contributing factors (Craig, 2008). A reasonable place to start is the examination of a genetic factor and the associated

phenotype. This association can be transferred into better knowledge of the disease mechanism, which is what leads to new or better treatment options.

AMD and AD are prime examples of complex diseases. Both have benefitted from the information gained from GWAS but have, so far, been studied separately in terms of the genetic factors and associated phenotypes. This study aims to investigate the AMD-cognitive impairment comorbidity with respect to possible common genetic factors.

Candidate Genes

The similar disease risk factors in AMD and AD and the common histopathology lead to the hypothesis that gene mutations may be the starting point of a common pathogenesis in these conditions. The first mutation to be associated with AMD was the Y402H SNP in CFH. This association was reported by four studies in 2005 (Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005; R J Klein et al., 2005). CFH is the gatekeeper for the complement cascade. A mutation impairing its function results in increased inflammation.

Inflammation was first associated with AD in 1907 by Alzheimer himself (Alzheimer, Stelzmann, Schnitzlein, & Murtagh, 1995). In addition, beta-amyloid (βA), the hallmark of both AMD and AD has been shown to trigger the complement cascade. Considering that complement-driven inflammation and βA are implicated in both AMD and AD, the same polymorphisms that infer risk for AMD may also modulate AD risk.

Second to CFHY402H, the SNP having the greatest impact on AMD risk is ARMS2A69S. Compared to CFH, ARMS2 is not as well characterized. Research to date has found that it is expressed in the brain and in the retina (Gatta et al., 2008). It also contributes to activation of the complement cascade. A recent study suggests ARMS2 is involved in complement-mediated clearance of cellular debris. The A69S SNP causes mRNA instability resulting in a deficiency of the protein. Without the ARMS2 protein present, the complement cascade is not activated to clean up necrotic cells and unwanted debris. This can lead to the formation of drusen and senile plaques (Micklisch et al., 2017).

FADS1 and Cognitive Health

To date, FADS1 rs174547 has not been investigated as a factor involved in both AMD and AD. The role of this SNP in AMD was discussed in Chapter Two. In terms of AD, the condition of the FADS1 gene product is important for the structural integrity of the brain. Approximately half of the brain's dry mass is composed of omega-3 PUFAs, the lipids that depend on FADS1 for their biosynthesis, and approximately 90% of this is DHA (Weiser, Butt, & Mohajeri, 2016). DHA is used in the phospholipid membranes of brain cells and also serves as a precursor for bioactive molecules required for brain function (Freemantle, Lalovic, Mechawar, & Turecki, 2012). It is enriched at synaptic terminals and changes in its concentration can affect cellular characteristics and physiological processes such as neurotransmitter release, signal transduction, neuroinflammation and neuronal differentiation and growth (Orr & Bazinet, 2008; Uauy & Dangour, 2006).

Altered brain PUFA content has been implicated in cognitive, psychiatric and neurodegenerative disorders (Fraser, Tayler, & Love, 2010; McNamara, Liu, Jandacek, Rider, & Tso, 2008; Muldoon et al., 2010). A study measuring dietary intake of DHA showed that baseline levels positively correlated with larger volumes of gray matter and better declarative memory performance (Titova, Sjögren, Brooks, & Benedict, 2013). An observational study relying on blood DHA levels over dietary intake suggested a positive correlation between DHA concentration and cognition in healthy adults (Muldoon et al., 2010). Further, a 5-year prospective longitudinal study showed that a decline in MMSE scores was negatively correlated with DHA levels (van Gelder, Tijhuis, Kalmijn, & Kromhout, 2007). This led researchers to theorize that lower levels of DHA are associated with unfavourable cognition (Freemantle et al., 2012; Lassek & Gaulin, 2011). When this theory was applied to treatment, it was found that adults with mild memory complaints supplemented with DHA and EPA showed improvement episodic memory (Yurko-Mauro et al., 2015). A randomized controlled trial in healthy older adults found improvements in executive function, white matter integrity, and neurovascular function as well as increases in gray-matter volume when supplemented with DHA over a period of 26 weeks (Weiser et al., 2016). The Alzheimer's Disease Neuroimaging Initiative Trail

also found significant correlations between fish oil supplementation and lower levels of brain atrophy in the hippocampus and cortical brain matter in patients (Hebert, Weuve, & Evans, 2013).

The hallmark neuropathologies of AD are the βA plaques seen on post-mortem examination. βA plaques limit brain plasticity, promoting the loss of memory by increasing inflammation via activated microglia and higher levels of pro-inflammatory cytokines (Weiser et al., 2016). *In vitro* studies have shown that DHA inhibits the interaction of β-secretase with APP, essentially stopping the formation of βA aggregates. β-secretase is the enzyme that processes APP to form βA, which cannot be cleared by microglia. The prevention of this interaction decreases the formation of βA and stimulates microglia to properly phagocytize APP products (Grimm et al., 2011; Hjorth et al., 2013).

Limitations of Recent Studies

A number of studies have reported an association between AMD and cognitive impairment. However, these studies have been limited regarding the evaluation of cognitive impairment. Some studies have used subscales of neuropsychological scales, such as the Wechsler Adults Intelligence Scale to assess cognitive function (AREDS Research Group, 2006). Alone, these subscales cannot be used to reach a clinical diagnosis of cognitive impairment. Many studies used the MMSE and found an association of cognitive impairment with late AMD, but not early AMD (Baker et al., 2009). There is evidence of this test not being sensitive to MCI (Dag et al., 2014; Hoops et al., 2009; Nasreddine et al., 2005). As a result, it is possible that earlier stages of AMD could be associated with milder cognitive impairment too subtle to be detected by the MMSE.

This study aimed to overcome these limitations by using the MMSE as well as the MoCA, which has been shown to be able to identify cases of MCI not detected by the MMSE (Dag et al., 2014). Additionally, psychophysical measures were incorporated to measure the processing ability of the brain that can affect visual perception.

Methods

Participants were recruited from the Montreal Retina Institute and the School of Optometry Clinic at the Université de Montréal. The study protocol was approved by *Le Comité d'éthique de la recherche en santé at the Université de Montréal* and followed the tenets of the Declaration of Helsinki. All study participants gave signed informed consent prior to their participation in the study.

Subjects aged 70 years or older and diagnosed with AMD by an ophthalmologist or optometrist were recruited for this study. Individuals with comorbid glaucoma, neurological disorders, or a diagnosis of dementia were excluded. The control group also consisted of participants aged 70 years or older. They were required to have normal, healthy retinas. Exclusion criteria included retinal disease, glaucoma and diagnosed cognitive problems.

For 10 participants from the patient group, genotyping was conducted as part of a previous study (Smailhodzic et al., 2012) by Radboud University Medical Center in Nijmegen, Netherlands. The remainder of the patient group and the control group were genotyped by targeted mutation analysis from saliva samples by Asper Biotech Ltd. in Estonia.

Cognitive Assessment

Cognitive testing incorporated a combination of two questionnaires, (the Mini-Mental State Exam (MMSE) and the Montreal Cognitive Assessment (MoCA), and psychophysical testing (the NeuroMinder and Neurotracker).

The MMSE is a brief test that screens for cognitive impairment. It is typically used to detect dementia, estimate severity of cognitive impairment, and monitor cognitive changes over time. It covers a number of categories including orientation to time and place, repeating lists of words, simple arithmetic, language use and comprehension, and basic motor skills. Customized versions of the MMSE exist. A version tailored to the

visually impaired population was used. (See Appendix C for a copy of the MMSE questionnaire and scoring instructions.)

The MoCA is used to screen for mild cognitive impairment. It covers several different cognitive aspects. The short-term-memory recall task involves two learning trials of five nouns and delayed recall after approximately 5 minutes. Multiple aspects of executive function are assessed using an alternation task, a phonemic fluency task, and a two-item verbal abstraction task. Attention, concentration and working memory are evaluated using a sustained attention task (target detection using tapping), a serial subtraction task and digits forward and backward. Language is assessed using a three-item naming task with low-familiarity animals (ex. lion, camel, rhinoceros) and repetition of two syntactically complex sentences. Finally, orientation to time and place is evaluated. A version of this questionnaire has also been modified for the visually impaired population. (See Appendix C for the version of the MoCA used and the corresponding scoring instructions.)

The NeuroMinder by *Cognisens*, is a psychophysical tool used to study the subtle effects of cognitive impairment as it relates to vision. It is used to measure mild perceptual impairment (MPI), the precursor to mild cognitive impairment (MCI). It was used to examine perceptual-cognitive skills such as perceptual processing and working memory capacity for visual stimuli. The Neurominder uses a series of gratings to calculate a perception threshold for first-order (FO) and second-order (SO) stimuli. A higher threshold score would indicate poorer perception. The tool has been used to evaluate second-order perceptual processing in athletes and in older adults compared to younger adults (Faubert, 2002; Habak & Faubert, 2000). Deficits in second-order processing are thought to be one of the initial signs of MCI (Habak & Faubert, 2000). These deficits are so subtle that they would not be identified by traditional questionnaires.

The Neurotracker was used to examine perceptual-cognitive skills such as awareness, focus and decision-making. It involves following targets through dynamic motion across a wide 3D projection. The Neurotracker uses a 3D multiple-object tracking (3DMOT) task. Briefly, the task requires participants to view 8 spheres inside a virtual

cube and to track the movement of three of them over 10 seconds. The spheres move on linear trajectories, colliding with one another and with the walls of the virtual cube. More details are discussed in Legault et al. (2012). The 3DMOT speed threshold protocol was used as an output measure (Faubert & Sidebottom, 2012). A higher speed threshold indicates better performance.

Analysis

The Mann-Whitney U test was used to compare the resultant ranks of cognitive measures between the AMD group and the control group. The same test was also used to compare means between carriers and non-carriers of each SNP of interest. Kruskal-Wallis was used to compare the results of cognitive tests across zygosity for each SNP. All calculations were conducted using SPSS software, version 20.0. and JASP version 0.8.1.2 (IBM Corp, 2011; JASP Team, 2017).

Results

The AMD group consisted of 21 individuals (4M, 17F) with an average age of 78.9 years (range: 71-92). The control group consisted of 18 individuals (6M, 12F) with an average age of 74.1 years (Range: 70-85). Descriptive statistics were presented in Chapter 2.

Genetic testing determined that there were 21 carriers of CFHY402H with 17 being homozygous. Of the AMD group, 10 were carriers with five of them being homozygous. There were also 21 carriers of ARMS2A69S with six being homozygous. Of the AMD group, 12 were carriers including nine homozygotes. See Chapter 2 for genetic distributions.

Cognitive Questionnaires

All 39 participants completed the MMSE. Two participants scored in the range of mild impairment. Those participants were both from the AMD group. One of them was heterozygous for the ARMS2A69S and homozygous for the FADS1 SNP while the other was homozygous for the FADS1 SNP.

There were 13 individuals scoring in the range of MCI according to the MoCA. There were eight from the AMD group (38.1%) and five from the control group (27.7%). Although more individuals from the AMD group scored positive for MCI, the average score between groups was not significantly different, $t(37) = -1.197$, $p = 0.239$ and $U = 150.0$, $p = 0.273$. The range of scores between groups did differ. From the control group, four of the five scored 25 points, one point below the normal range. The fifth individual scored 24 points. The eight from the AMD group had a broader range in scores from 20 to 25.

Although the average MoCA scores did not differ between the AMD group and controls, the subscales they had difficulty with did. Those from the control group scoring positive for MCI had significantly lower scores on the delayed recall subscale compared to those from the same group who passed, $U = 2.5$, $p = 0.002$. Comparatively, those from the AMD group with MCI scored significantly poorer on delayed recall, $U = 14.5$, $p = 0.005$, in addition to the orientation, $U = 37.5$, $p = 0.034$, and abstraction, $U = 24.5$, $p = 0.007$, subscales of the MoCA compared to the rest of the AMD group.

When scores for the blind version of the MoCA were calculated, the number of individuals scoring in the MCI range decreased from 13 to 10. Only one of these three individuals had AMD. Results changed little for the AMD group with 33.3% scoring positive for MCI on the MoCA Blind, while there was an improvement for the control group with 16.7% scoring positive for MCI. See Table XXI and Figure 13 for details of the results of the cognitive questionnaires.

The CFHY402H SNP was carried by seven of the 13 who scored below normal on the MoCA. Two of them were homozygotes, neither of which had AMD. Scores obtained by carriers and non-carriers of CFHY402H were not significantly different. In terms of the subscales, carriers of the SNP had significantly lower scores on only the

Table XXI. Cognitive Questionnaire Results

AMD Group	MoCA	MoCA Blind	MMSE	Control Group	MoCA	MoCA Blind	MMSE
AMD001	27	19	30	CTRL001	30	22	30
AMD002	25*	17*	28	CTRL002	29	21	30
AMD003	24*	16*	29	CTRL003	27	19	27
AMD004	28	20	29	CTRL004	24*	17*	28
AMD005	24*	17*	29	CTRL005	26	19	27
AMD006	27	19	26	CTRL006	29	22	30
AMD007	20*	14*	24*	CRTL007	29	21	30
AMD009	25*	18	25*	CRTL008	29	21	30
AMD010	26	18	29	CRTL009	28	20	30
AMD011	27	20	27	CRTL010	25*	17*	27
AMD012	30	22	30	CTRL011	29	22	30
AMD013	30	22	30	CTRL012	25*	17*	30
AMD014	29	21	30	CTRL013	30	22	30
AMD015	28	21	30	CTRL014	27	19	30
AMD016	28	20	29	CTRL015	25*	19	30
AMD017	28	22	29	CTRL016	25*	18	30
AMD018	24*	16*	27	CTRL017	29	22	30
AMD019	28	22	29	CTRL018	30	22	30
AMD020	29	22	30				
AMD022	30	22	30				
AMD023	22*	16*	29				

** participants scoring positive for MCI*

Note: The MoCA is scored out of 30, with scores below 26 considered in the range of MCI. The MoCA Blind is scored out of 22 with scores below 18 considered in the range of MCI. The MMSE is scored out of 30 with scores below 25 considered in the range of cognitive impairment. See Appendix C for further details of questionnaires and scoring.

	Test	Statistic	df	p	Cohen's d	95% CI	
						Lower	Upper
MoCA score	Student	-1.197	37	0.239	-0.385	-2.521	0.648
	MWU	150.00		0.273	-0.385	-2.000	1.000

Descriptive Statistics

	Group	N	Mean	SD	SE
MoCA Score	AMD	21	26.62	2.711	0.592
	Control	18	27.56	2.064	0.487

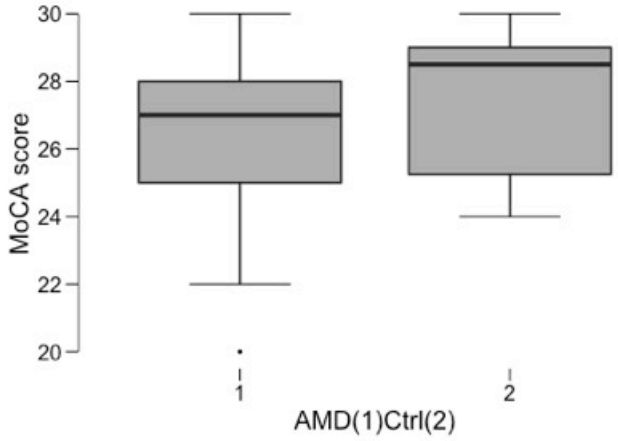

Figure 13. The Results of the MoCA: AMD Group vs. Control Group

The independent Student's t test showed no significant difference between mean MoCA scores of the AMD group versus the control group. The same was true when the nonparametric Mann-Whitney U test (MWU) was used to compare mean ranks. The box plot displays the AMD group (1) and control group (2) on the x-axis versus the MoCA score on the y-axis.

language subscale, $H = 6.27$, $p = 0.044$. The effect was more pronounced in carriers with AMD.

The ARMS2 SNP was carried by five of the individuals who scored below the normal range on the MoCA. They were all heterozygous for the SNP, with four of them being from the AMD group and one from the control group. MoCA scores did not significantly differ between carriers and non-carriers of ARMS2A69S. The same was true for subscale scores.

All 13 of those scoring positive for cognitive impairment on the MoCA were carriers of the FADS1 SNP. Seven of the eight from the AMD group with MCI were homozygotes, while three of the five from the control group were homozygotes. Kruskal-Wallis showed that homozygous carriers of the FADS1 SNP had lower cognitive scores compared to heterozygous carriers and non-carriers, $H = 8.52$, $p = 0.014$. Homozygotes with AMD had particular difficulty on the language and abstraction subscales.

Neurominder and 3DMOT

There were no significant correlations between the Neurominder or 3D-MOT results and results of the cognitive questionnaires. There were also no correlations with age. The Mann-Whitney U test showed no significant differences between Neurominder or 3D-MOT thresholds and carrier status of any of the SNPs of interest. However, when zygosity was considered, scores on FO orientation and direction thresholds as measured by the Neurominder significantly differed with respect to ARMS2 SNP zygosity, $F(2,34) = 3.479$, $p = 0.042$ and $F(2,34) = 5.230$, $p = 0.010$, respectively. Non-carriers had lower detection thresholds, indicating better performance on the test compared to heterozygotes or homozygotes, who had the highest thresholds (Figures 14 & 15).

Independent Student's t tests and Mann-Whitney U tests showed significant differences between the performance of individuals in the AMD group and controls on both the Neurominder and 3D-MOT. The control group had significantly lower detection

ANOVA

	Sum of Squares	df	Mean Square	F	p
ARMS2A69S	0.166	2	0.083	3.479	0.042

Post Hoc - Bonferroni

Zygosity		Mean Difference	SE	t	p
0	1	-0.057	0.054	-1.064	0.883
	2	-0.191	0.073	-2.631	0.037
1	2	-0.134	0.075	-1.798	0.242

Descriptive Statistics

Zygosity	Mean (FO direction)	SD	N
0	-1.975	0.137	18
1	-1.918	0.171	15
2	-1.784	0.162	6

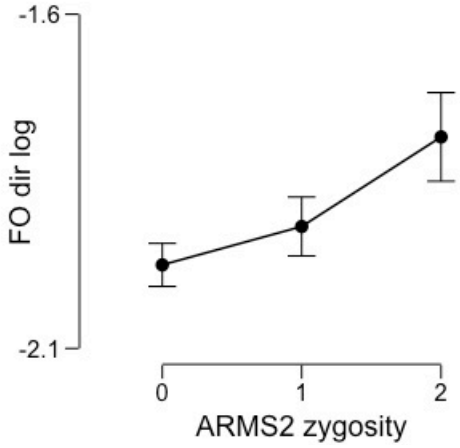

Figure 14. FO Direction and ARMS2A69S Zygosity.

One-way ANOVA showed a significant difference between FO direction perception thresholds based on ARMS2A69S zygosity. Post-hoc analysis determined homozygotes had a significantly higher detection threshold (poorer score) compared to non-carriers. The graph plots zygosity along the x-axis versus the log threshold of perception for FO direction. The bars display the standard error for non-carriers (0), heterozygotes (1) and homozygotes (2).

ANOVA

	Sum of Squares	df	Mean Square	F	p
ARMS2A69S	0.404	2	0.202	5.230	0.010

Post Hoc - Bonferroni

Zygosity		Mean Difference	SE	t	p
0	1	-0.029	0.069	-0.419	1.000
	2	-0.293	0.093	-3.160	0.010
1	2	-0.264	0.095	-2.781	0.026

Descriptive Statistics

Zygosity	Mean (FO orientation)	SD	N
0	-1.679	0.166	18
1	-1.650	0.210	15
2	-1.386	0.246	6

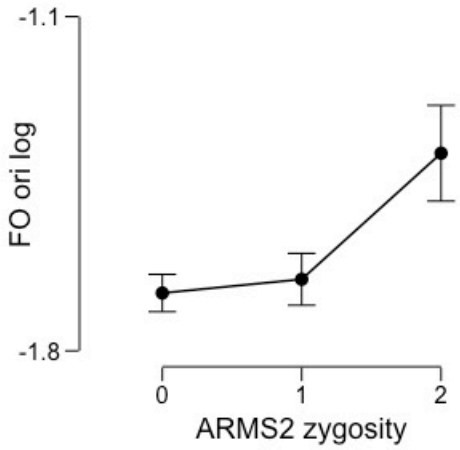

Figure 15. FO Orientation and ARMS2A69S Zygosity.

One-way ANOVA showed a significant difference in FO orientation perception thresholds based on ARMS2A69S zygosity. Post-hoc analysis determined homozygotes had a significantly higher detection threshold (poorer score) compared to non-carriers and herterozygotes. The graph plots zygosity along the x-axis versus the log threshold of perception for FO orientation. The bars display the standard error for non-carriers (0), heterozygotes (1) and homozygotes (2).

thresholds for first order direction (Figure 16), $t(37) = 4.282$, $p < 0.001$ and $U = 324.5$, $p < 0.001$, and orientation (Figure 17), $t(37) = 3.058$, $p = 0.004$ and $U = 292.0$, $p = 0.004$, stimuli compared to the AMD group. The same was true for second order direction (Figure 18), $t(37) = 3.336$, $p = 0.003$ and $U = 296.5$, $p = 0.003$, and orientation (Figure 19), $t(37) = 2.950$, $p = 0.005$ and $U = 279.5$, $p = 0.011$, stimuli. The speed threshold on the 3D-MOT was significantly higher for the control group than for the AMD group (Figure 20), $t(37) = -3.963$, $p < 0.001$ and $U = 50.5$, $p < 0.001$.

Discussion

The results of this study supported with those of Dag *et al.* (2014) showing that the MoCA is more sensitive to cases of MCI than the MMSE. Most of the participants had nearly perfect scores on the MMSE while there was greater variation on the MoCA. When scores on the original MoCA were compared to their MoCA Blind scores, there was not a lot of change in terms of those from the AMD group that passed or failed. This was surprising, since a study on the sensitivity and specificity of the MoCA Blind determined that removing the visual items resulted in a better specificity for detecting MCI (Wittich et al., 2010). This is likely due to sampling. The current sample was recruited from retina practices and optometry clinics, resulting in a wider range of visual acuities and fixation stabilities. Wittich and his colleagues recruited their sample from a rehabilitation centre requiring its clients to have a visual acuity of 20/60 in the better eye. It would be useful to determine at which visual acuity the MoCA Blind is more accurate. Also, fixation stability may be a factor to consider in whether the visual components of a cognitive questionnaire should be used in evaluating someone with vision impairment given that it has an impact on activities of daily living such as face recognition (Seiple et al., 2013), reading speed (Seiple et al., 2005) and eye-hand coordination (Timberlake et al., 2008).

The percentage of those with AMD who did not pass the MoCA was in agreement with previous studies (Duponsel et al., 2010; Wittich, Murphy, & Mulrooney, 2014) at 38.1%. The percentage of controls scoring positive for MCI on the MoCA (27.7%) was

	Test	Statistic	df	p	Cohen's d	95% CI	
						Lower	Upper
FO direction	Student	4.284	37	<0.001	1.376	0.099	0.276
log score	MWU	324.50		<0.001	1.376	0.078	0.245

Descriptive Statistics

	Group	N	Mean	SD	SE
FO direction log Score	AMD	21	-1.837	0.138	0.030
	Control	18	-2.024	0.133	0.031

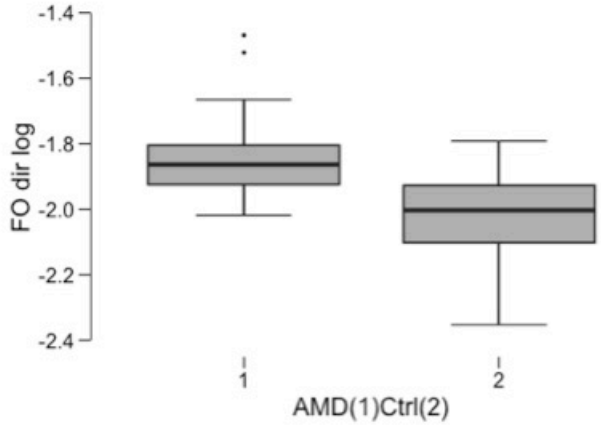

Figure 16. FO Direction Results from the Neurominder.

An Independent Student's t Test showed a significant difference in mean perception thresholds for FO direction thresholds between the AMD group and control group. This result remained unchanged when the nonparametric Mann-Whitney U test was used to compare mean ranks. The AMD group had a higher detection threshold (poorer score) compared to the control group. The boxplot displays the log perception threshold for FO direction (y-axis) for the AMD group (1) and control group (2).

	Test	Statistic	df	p	Cohen's d	95% CI	
						Lower	Upper
FO orientation	Student	3.058	37	0.004	0.982	0.065	0.321
log score	MWU	292.00		0.004	0.982	0.040	0.284

Descriptive Statistics

	Group	N	Mean	SD	SE
FO orientation log Score	AMD	21	-1.534	0.228	0.050
	Control	18	-1.727	0.153	0.036

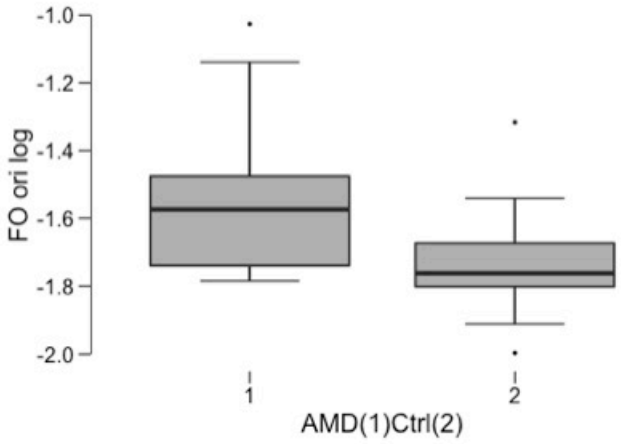

Figure 17. FO Orientation Results from the Neurominder.

An Independent Student's t test showed a significant difference in mean FO orientation perception thresholds between the AMD group and the control group. The Mann-Whitney U test results were in agreement. The AMD group had a higher detection threshold (poorer score) compared to the control group. The boxplot displays the average log perception threshold for FO orientation (y-axis) for the AMD group (1) and control group (2).

	Test	Statistic	df	p	Cohen's d	95% CI	
						Lower	Upper
SO direction	Student	3.336	37	0.003	1.072	0.120	0.490
log score	MWU	296.50		0.003	1.072	0.112	0.468

Descriptive Statistics

	Group	N	Mean	SD	SE
SO direction log Score	AMD	21	-0.470	0.254	0.055
	Control	18	-0.775	0.316	0.075

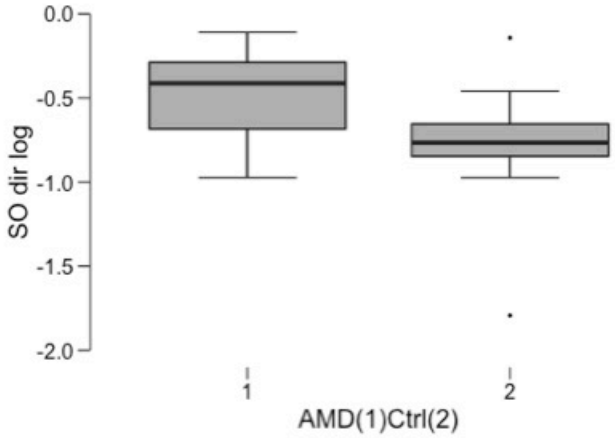

Figure 18. SO Direction Results from the Neurominder.

An Independent Student's t test showed a significant difference in mean SO direction perception thresholds between the AMD group and the control group. The Mann-Whitney U test of mean ranks supported this result. The AMD group had a higher detection threshold (poorer score) compared to the control group. The boxplot displays the average log perception threshold for SO direction (y-axis) for the AMD group (1) and control group (2).

	Test	Statistic	df	p	Cohen's d	95% CI	
						Lower	Upper
SO orientation	Student	2.950	37	0.005	0.948	0.071	0.381
log score	MWU	279.50		0.011	0.948	0.072	0.403

Descriptive Statistics

	Group	N	Mean	SD	SE
SO orientation log Score	AMD	21	-0.678	0.271	0.059
	Control	18	-0.904	0.193	0.045

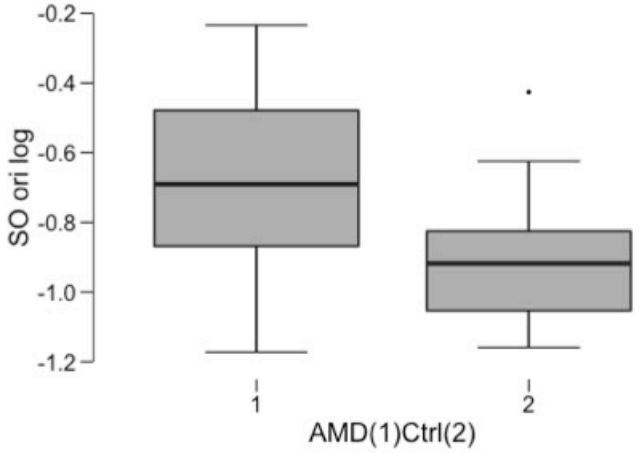

Figure 19. SO Orientation Results from the Neurominder

An Independent Student's t test showed a significant difference in mean SO orientation perception thresholds between the AMD group and the control group. The Mann-Whitney U test was in agreement with this result. The AMD group had a higher detection threshold (poorer score) compared to the control group. The boxplot displays the log perception threshold for SO orientation (y-axis) for the AMD group (1) and control group (2).

	Test	Statistic	df	p	Cohen's d	95% CI	
						Lower	Upper
3DMOT	Student	-3.936	37	<0.001	-1.264	-0.954	-0.306
log score	MWU	50.50		<0.001	-1.264	-0.825	-0.233

Descriptive Statistics

	Group	N	Mean	SD	SE
3DMOT log Score	AMD	21	-0.829	0.648	0.141
	Control	18	-0.199	0.215	0.051

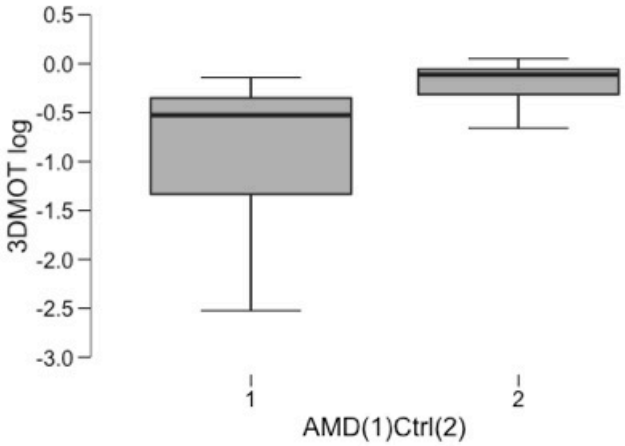

Figure 20. 3DMOT Results in the AMD Group vs. Control Group

An Independent Student's t test showed a significant difference in mean 3DMOT threshold speeds between the AMD group and the control group. The Mann-Whitney U test also supported this. The AMD group had a lower speed threshold (poorer score) compared to the control group. The boxplot displays the log perception threshold for correction identification on the 3DMOT (y-axis) for the AMD group (1) and control group (2).

high compared to MCI prevalence reported elsewhere (Gauthier et al., 2006; Lopez et al., 2003). This is likely due to sampling error and would be lower in a larger sample. In addition to size, another limitation related to the sample is the character of the individuals who are likely to volunteer for such a study. Given the specialized equipment this research required, testing had to be conducted onsite. This was a factor in the decision of whether or not to participate for many individuals. Those less confident in their independence and mobility were more likely to decline participation causing a potential bias in the sample. Although a decline due to cognitive impairment would affect the groups equally, this bias would likely be stronger in the AMD group due to the confounding factor of functional vision impairment. This bias could be overcome by using portable equipment should it ever become available.

MoCA scores were not significantly different between the AMD group and the control group, but the groups did differ on which subscales were difficult for them. Those from the control group who did not pass the MoCA had difficulty with delayed recall, which is typical of an MCI diagnosis. Not all cases of MCI progress to AD. Prospective research has shown that cases of MCI presenting with deficits in memory in addition to deficits in other cognitive domains are more likely to convert to AD (Summers & Saunders, 2012). Those with AMD who did not pass the MoCA had difficulty on the delayed recall subscale in addition to orientation and abstraction. This leads to the hypothesis that those with AMD scoring positive for MCI on the MoCA may be at a higher risk of developing AD compared to controls. Prospective studies would have to be conducted to confirm this.

The CFHY402H and ARMS2A69S SNPs appeared not to have an impact on the results of cognitive questionnaires, as MoCA scores were not significantly different between carriers and non-carriers. Conversely, all participants scoring positive for MCI on the MoCA were carriers of FADS1 rs174547 with homozygotes having the lowest scores. This finding supports the biochemical research discussed in the introduction. The presence of the rs174547 SNP increases delta-5 desaturase activity which, in turn, reduces DHA (Cakiner-Egilmez, 2008; Fauser et al., 2011; Hellstrand et al., 2012; Merino et al., 2011), a vital component for brain structure and cognition (Freemantle et

al., 2012; Lassek & Gaulin, 2011; van Gelder et al., 2007). A direct link should still be established, but these results support the theory of FADS1 rs174547 playing a role in cognitive impairment.

In terms of the psychophysical measures, the control group outperformed the AMD group on every parameter. This was expected as vision obviously plays a role in the measurement of visual perception. (The aspects of vision that affected the AMD group's performance will be discussed in a later chapter.) Interestingly, there were no significant differences on Neurominder or Neurotracker scores based on those who passed or failed the MoCA. When the AMD group and control group were analysed separately, there was still nothing of interest to report for the AMD group, but the control group showed significant correlations between MoCA score and 3DMOT log speed threshold and SO orientation log threshold. In other words, control subjects with better MoCA scores were able perform better on the 3DMOT task and detection of SO orientation on the Neurominder.

Carriers of the ARMS2A69S SNP appear to have poorer performance on the FO detection tasks from the Neurominder, especially homozygotes. The FO tasks were based on the modulation of contrast in the gratings presented to the participant. Contrast sensitivity typically depends on the integrity of photoreceptors in the retina. Photoreceptor dysfunction due to the presence of the ARMS2 SNP could account for the poor performance of homozygous carriers. Expression studies on ARMS2 have localized the gene product to the photoreceptors (Gatta et al., 2008). Although its exact function is still under investigation, the A69S SNP is thought to prevent the production of the ARMS2 gene product.

Conclusion

Although the prevalence of MCI among those with AMD was not much higher than controls in this sample, the prevalence is higher than that reported in other normally-sighted populations (Gauthier et al., 2006). Additionally, those with AMD scoring positive for MCI according to the MoCA had difficulty with different cognitive domains

compared to controls scoring positive for MCI. This distribution of cognitive impairment indicates that those with AMD and MCI may be more likely to progress to AD than controls with MCI.

Psychophysical measures of perception like the Neurominder and 3DMOT appear to provide more insight on cognitive impairment in healthy older adults as their performance correlated with outcomes from cognitive questionnaires. Measures like these are too dependent on visual information to provide accurate insight on the cognitive status of individuals with AMD. This is evidenced by the differences in performance of the AMD group versus the controls and the lack of correlation between performance and cognitive questionnaire scores in the AMD group.

No significant relationships between the most prominent AMD SNPs, CFHY402H and ARMS2A69S, and MCI were identified, giving support to previous claims that although AMD and AD have many similarities, the underlying genetic mechanisms are different (Proitsi et al., 2012). Findings were different for the FADS1 SNP. Carriers, both with and without AMD, were more likely to have lower cognitive scores compared to non-carriers. Further, all those scoring positive for MCI according to the MoCA were homozygous for the FADS1 SNP. These results suggest FADS1 rs174547 may be a better focus for better understanding any common genetic mechanism in the AMD-MCI co-morbidity.

Chapter 6: Study 5 - Retinal Structure, Visual Function, and Cognition

A growing body of literature has been reporting a co-occurrence of visual and cognitive impairment (Baker et al., 2009; Boxerman et al., 2015; Harrabi et al., 2015; Pham et al., 2006; Whitson et al., 2010; T. Y. Wong et al., 2002). Researchers in the field of low vision rehabilitation have had to adapt cognitive measures to accommodate vision impairment (Busse et al., 2002; Nasreddine et al., 2005) and conversely, low vision rehabilitation programs are being designed to take cognitive impairment into account (Whitson et al., 2011, 2013). Several studies have investigated the ocular manifestations of cognitive impairment, either to better define the visual complaints from AD patients or in search for biomarkers for a definitive diagnosis.

Researchers testing functional vision in AD patients have identified a reduced adaptive reflex in response to light. Idiaquez and colleagues found a smaller resting pupil diameter, a smaller darkness reflex amplitude and a slower dilation velocity in individuals with AD (Idiaquez, Alvarez, Villagra, & San Martin, 1994). A later study investigated pupillary response to Tropicamide in AD, Parkinson's disease and control. The effect of Tropicamide was not significantly different between groups, however, they did find that peak constriction amplitude to the pupillary light reflex was significantly reduced in both disease groups. Further, the peak constriction amplitude significantly correlated with the severity of dementia in the AD group (Granholm et al., 2003).

Some research groups were more focused on ocular anatomy. A 2010 study used a scanning laser ophthalmoscope (SLO) to observe changes in the optic nerve head of patients with Alzheimer's disease (AD) compared to controls. They reported a decrease in the volume and area of the neuroretinal rim and a higher cup to disc ratio in the AD group compared to controls (Hinton et al., 2010). These findings led the research team to hypothesize that AD was associated with decline in optic nerve fibers passing through the optic nerve head into the brain. This hypothesis is supported by earlier reports of optic disc cupping and pallor (Danesh-Meyer et al., 2006; Hinton et al., 1986, 2010).

Since beta-amyloid, better known as the hallmark of AD, was identified as a component of some drusen in AMD (Dentchev et al., 2003; L V Johnson et al., 2002), there has been a greater focus on the retina being a link between AMD and AD. Changes in the retinal nerve fiber layer (RNFL) were examined in AD patients versus controls. Although some studies report no differences in RNFL thickness between individuals with AD and age-matched controls (Kergoat, Kergoat, Justino, Chertkow, et al., 2001), many found a significant thinning in the RNFL of those with AD (Iseri et al., 2006; Parisi et al., 2001). Others reported similar results including a MCI group, which had greater RNFL thickness compared to the AD group, but still thinner than controls (Kesler et al., 2011; Paquet et al., 2007). Based on post-mortem histological findings (J C Blanks et al., 1981, 1987; Hinton et al., 1986), it is thought this decrease in thickness is due to retinal ganglion cell degeneration (Danesh-Meyer et al., 2006).

More recently, researcher have been attempting to find a way of identifying beta-amyloid in the live retina. A Canadian research group has been attempting to identify the spectral signature of beta-amyloid through two-photon microscopy (Avila et al., 2015; Campbell et al., 2015; Hamel et al., 2016) while an American group used a curcumin formulation to image beta-amyloid *in vivo* in rats (Koronyo-Hamaoui et al., 2011). The latter group recently published a proof of concept trial in humans. They were able to visualize the load and distribution of beta-amyloid in the retina via OCT, but found no correlation between retinal amyloid index and Mini Mental State Exam Scores.

The aim of this section of the study was to reinforce the connections drawn between AMD, the retina, and cognitive impairment through the presence of drusen. This was accomplished through the use of functional vision parameters such as fixation stability and eccentricity, which are now standard in the evaluation of visual function in AMD and more sensitive cognitive measures than that of the MMSE. There was an attempt to establish links between drusen characteristics observed on OCT and cognitive impairment without the use of specialized equipment that is unavailable clinically, or fluorochromes used in other studies.

Methods

Participants were recruited from the Montreal Retina Institute and the School of Optometry Clinic at the University of Montreal. Study protocol was approved by Le Comité d'éthique de la recherche en santé at the Université de Montréal and followed the tenets of the Declaration of Helsinki. All study participants gave signed informed consent prior to their participation in the study.

Subjects aged 70 years or older and diagnosed with AMD by an ophthalmologist or optometrist were recruited for this study. Individuals with comorbid glaucoma, neurological disorders or a diagnosis of dementia were excluded. The control group consisted of participants aged 70 years or older. They were required to have normal, healthy retinas. Exclusion criteria included retinal disease, glaucoma and diagnosed cognitive problems.

Visual Function

Visual function was evaluated using a variety of parameters. This included distance visual acuity, contrast sensitivity, colour vision, retinal sensitivity, location of fixation and fixation stability. (See Appendices B - D for further description.)

Retinal sensitivity was measured using the microperimetry function of the Optos Optical Coherence Tomographer/Scanning Laser Ophthalmoscope (OCT/SLO). A polar 3 12° grid was centred on the fovea. The stimulus was equivalent to a Goldman III with 200ms projection time. A 4-2 strategy was used with an automatic eye tracker to compensate for eye movements.

Fixation stability was measured using the SLO function that uses confocal scanning laser microscopy to view the retinal surface in real time. The operator is able to view a participant’s retina as he/she looks at a projected image. This allows identification of the location of fixation on the retina and its stability.

The fixation stability task required the participant to gaze as steadily as possible at a target for a period of 20 seconds with a sampling rate of 4Hz. The final output of this test was the superimposition of all the photos taken during the 20-second time frame. In the case of stable fixation, the target crosses are clustered together on the retina while unstable fixation would display the crosses spread out. Fixation stability was quantified using a bivariate contour ellipse area (BCEA). This is the area in which 95% of the target crosses are located, measured in square degrees. A smaller area was indicative of better fixation stability.

The location of fixation or preferred retinal locus (PRL) was determined using the results of the fixation stability test with the retinal topography function of the OCT/SLO. Retinal topography measures the volume of the retinal layers. Such a scan allows the identification of the anatomical fovea. Once the fovea is located, the image can be superimposed over the result of the fixation test and the distance between the anatomical fovea and the PRL can be measured.

Retinal Structure

Retinal structure was evaluated using fundus photography and the OCT/SLO.

Colour fundus photos were taken using a Canon CR-1 fundus camera. Two optometrists evaluated photos using the Age-related Eye Disease Study (AREDS) grading schema.

The Optos OCT/SLO raster scan function was used to take cross sectional images of the retina in each eye. A total of 32 parallel cross-sectional scans are taken from the top of the fundus to the bottom. Raster scans were used to identify drusen characteristics based on four categories: shape, homogeneity, reflectivity and hyper-reflective foci.

MATLAB was used to measure drusen area and tally the number of drusen falling under each characteristic category and subcategory.

Cognitive Assessment

Cognitive testing incorporated a combination of questionnaires (the Mini-Mental State Exam (MMSE) and the Montreal Cognitive Assessment (MoCA) and psychophysical testing (Neurominder and Neurotracker).

The MMSE is a brief test that screens for cognitive impairment. It is typically used in medicine to detect dementia, estimate severity of cognitive impairment and monitor cognitive changes over time. It covers a number of categories including orientation to time and place, short-term memory, simple arithmetic skills, language use and comprehension, and basic motor skills.

The MoCA is used to screen for mild cognitive impairment. It also covers several different cognitive aspects. The short-term memory recall task involves two learning trials of five nouns and delayed recall after approximately 5 minutes. Multiple aspects of executive functions are assessed using an alternation task, a phonemic fluency task, and a two-item verbal abstraction task. Attention, concentration and working memory are evaluated using a sustained attention task (target detection using tapping), a serial subtraction task and digits forward and backward. Language is assessed using a three-item naming task with low-familiarity animals (ex. lion, camel, rhinoceros) and repetition of two syntactically complex sentences. Finally, orientation to time and place is evaluated. A version of this questionnaire has also been modified for the visually impaired population.

The Neurominder by *Cognisens*, is a psychophysical tool used to study the subtle effects of cognitive impairment as it relates to vision. It is used to measure mild perceptual impairment (MPI), the precursor to mild cognitive impairment (MCI). It was used to examine perceptual-cognitive skills such as perceptual processing and working memory capacity for visual stimuli. The Neurominder has been used to evaluate second-order perceptual processing in athletes and in older adults compared to younger adults. Deficits in second-order processing are thought to be one of the initial signs of MCI (Faubert, 2002; Habak & Faubert, 2000). These deficits are so subtle that they would not be identified by traditional questionnaires.

The Neurotracker was used to examine perceptual-cognitive skills such as awareness, focus and decision-making. It involves following targets through dynamic motion across a wide 3D projection. (See Appendix C).

Analysis

Pearson's correlation was used to compare continuous and interval data in visual function, retinal structure, and cognitive function.

Results

Visual Function – Cognitive Measures

When cognitive parameters were compared to those of visual function, Pearson's correlation showed a significant relationship between cognitive scores on the MoCA or MMSE and age, AMD onset, VA and Mars contrast sensitivity, but when the results were controlled for age, these correlations became non-significant. The Mann-Whitney U test showed no significant difference in visual function between those who passed the MoCA and those who failed. When the same analysis was repeated for the AMD group alone and for the control group alone, the same result was achieved in each case.

Retinal Structure – Visual Function

Drusen characteristics from the better eye, as identified on OCT images, did not correlate with visual acuity in the respective eye or binocular visual acuity. There were also no significant correlations with fixation stability (BCEA) or eccentricity in the best eye. Binocular contrast sensitivity was negatively correlated with concave drusen, $r = -0.515$, $p = 0.024$, drusen of high reflectivity, $r = -0.478$, $p = 0.039$, and drusen of mid reflectivity in the better eye, $r = -0.502$, $p = 0.029$.

Drusen characteristics in the worse eye did not correlate with contrast sensitivity or eccentricity, but some did correlate with visual acuity and fixation stability. Visual acuity in the worse eye was positively correlated with the presence of core, $r = 0.506$, $p = 0.032$, and overlying hyper-reflective foci, $r = 0.590$, $p = 0.010$. Binocular visual acuity

also significantly correlated with some drusen characteristics in the worse eye. These included drusen area, convex drusen, concave drusen, presence of core and presence of overlying hyperreflective foci. Fixation stability (log BCEA) was positively correlated with drusen area, drusen that were concave or convex in shape, drusen of high reflectivity, overlying foci, and drusen of nonhomogeneous content with or without core. These correlations can be seen in Tables XXII - XXVII.

Retinal Structure – Cognitive Measures

The scores from cognitive questionnaires did not correlate with any of the drusen characteristics from OCT images. Neither the Mann-Whitney U test nor the independent Student's *t* test showed any significant difference between the presence of drusen characteristics in those that passed the MoCA and those who scored positive for MCI. The drusen characteristic that came the closest to reaching significance was overlying hyperreflective foci, $U = 15.50$, $p = 0.067$; $t(15.5) = 2.13$, $p = 0.050$.

In the better eye, the presence of drusen of mid reflectivity was positively correlated with detection thresholds on the Neurominder. Participants with more drusen of mid reflectivity had a poorer performance on the Neurominder compared to those with fewer mid reflective drusen. Additionally, the SO direction detection threshold was positively correlated with drusen area, concave drusen and drusen of high reflectivity, while the SO orientation detection threshold was positively correlated with only concave drusen. Threshold speed on the 3DMOT was not correlated with any drusen characteristics in the better eye (Tables XXVIII - XXX).

The 3DMOT had more numerous significant correlations with drusen characteristics in the worse eye. 3DMOT speed threshold negatively correlated with drusen of mid reflectivity, drusen with core, and drusen with overlying hyper-reflective foci. Participants with better performance on the 3DMOT had fewer drusen with mid reflectivity, cores, and overlying foci (Tables XXXI - XXXIII). There were fewer significant correlations with drusen characteristics from the worse eye and the Neurominder. SO direction detection thresholds were positively correlated with drusen of

low reflectivity and FO orientation detection thresholds were positively correlated with overlying hyper-reflective foci (Tables XXXXI - XXXIII).

Discussion

Visual Function - Cognitive Measures

It is not surprising that traditional parameters of visual function did not correlate with cognitive measures. Visual function, as measured by visual acuity and contrast sensitivity, is all related to the working capacity of the eye, which is diminished in AMD. It is important to remember that visual function is distinct from cognition. Visual input is part of perception and cognition, but it only provides physical information. Perception and cognition are higher order processes that use sensory input, but also take memory and experience into account. When vision is impaired, as in AMD, incorrect visual information can be delivered to the brain, but the brain still has memory and experience to sort this out.

Visual function did appear to have an impact on the results of the Neurominder and the 3DMOT. These instruments rely more heavily on visual cues compared to cognitive questionnaires like the MoCA and MMSE. Orientation tasks in particular, are contrast-defined and contrast sensitivity is known to be diminished in AMD as evidenced in this study and others. In the case of these instruments, experience and memory cannot be used to augment any incorrect visual information being received.

Retinal Structure – Visual Function

Research on binocular vision in AMD has shown that the eye with better visual acuity and oculomotor control drives binocular vision (Tarita-Nistor, Brent, Steinbach, & Gonzalez, 2011). The current study defined the better and worse eye of participants based on this definition. Binocular contrast sensitivity was negatively correlated with concave drusen and drusen of high and mid reflectivity in the better eye, which indicates binocular contrast sensitivity was poorer with greater amounts of those drusen characteristics.

Table XXII. Correlation of Visual Function with Drusen Characteristics in the Better Eye.

		Drusen Area	Convex	Concave	Pointy	Foci
LogMAR VA	*r*	0.126	-0.135	0.215	-0.112	0.218
	p	0.607	0.580	0.377	0.647	0.371
LogMAR VA OU	*r*	0.213	-0.076	0.296	-0.194	0.341
	p	0.382	0.757	0.219	0.427	0.153
Mars CS	*r*	-0.039	0.202	-0.127	-0.066	0.055
	p	0.875	0.406	0.605	0.788	0.824
Mars CS OU	*r*	-0.425	0.111	-0.515**	-0.194	-0.378
	p	0.069	0.651	0.024	0.425	0.110
Log BCEA	*r*	0.271	-0.107	0.366	-0.106	0.334
	p	0.277	0.673	0.135	0.676	0.176
Log Eccentricity	*r*	0.260	-0.121	0.359	-0.098	0.349
	p	0.298	0.631	0.143	0.698	0.155

$^{**}p < 0.05$, $^{***}p < 0.001$

There was a significant negative correlation between binocular contrast sensitivity and drusen that were considered concave in shape in the better eye. Having concave drusen in the better eye tended to be related to poorer contrast sensitivity.

Table XXIII. Correlations of Visual Function and Drusen Reflectivity in the Better Eye.

		High Reflectivity	Mid Reflectivity	Low Reflectivity
LogMAR VA	*r*	0.170	0.213	-0.122
	p	0.488	0.382	0.619
LogMAR VA OU	*r*	0.221	0.300	-0.049
	p	0.364	0.211	0.843
Mars CS	*r*	-0.128	-0.117	0.206
	p	0.602	0.634	0.398
Mars CS OU	*r*	-0.478**	-0.502**	0.073
	p	0.039	0.029	0.766
Log BCEA	*r*	0.333	0.298	-0.080
	p	0.177	0.230	0.751
Log Eccentricity	*r*	0.335	0.279	-0.096
	p	0.175	0.263	0.705

$^{**}p < 0.05$, $^{***}p < 0.001$

There were significant negative correlations between contrast sensitivity in the better eye and the presence of high and mid reflective drusen in the same eye. The greater the number of high or mid reflective drusen in the better eye, the poorer was the contrast sensitivity.

Table XXIV. Correlation of Visual Function with Drusen Content in the Better Eye.

		Homogeneous	Nonhomogeneous	Core
LogMAR VA	*r*	-0.128	0.117	0.115
	p	0.602	0.634	0.640
LogMAR VA OU	*r*	-0.056	0.173	0.254
	p	0.821	0.479	0.293
Mars CS	*r*	0.319	-0.094	0.123
	p	0.183	0.702	0.615
Mars CS OU	*r*	0.101	-0.397	-0.337
	p	0.680	0.093	0.158
Log BCEA	*r*	-0.135	0.262	0.184
	p	0.593	0.293	0.464
Log Eccentricity	*r*	-0.156	0.250	0.187
	p	0.538	0.318	0.457

$^{**}p < 0.05$, $^{***}p < 0.001$

There were no significant correlations with visual function and drusen content as identified on OCT.

Table XXV. Correlation of Visual Function with Drusen Characteristics in the Worse Eye.

		Drusen Area	Convex	Concave	Pointy	Foci
LogMAR VA	*r*	0.184	0.136	0.197	-0.139	0.590***
	p	0.464	0.592	0.433	0.583	0.010
LogMAR VA OU	*r*	0.572**	0.509**	0.562**	0.030	0.720***
	p	0.013	0.031	0.015	0.906	<0.001
Mars CS	*r*	-0.161	-0.330	-0.149	0.193	-0.354
	p	0.525	0.182	0.555	0.442	0.150
Mars CS OU	*r*	-0.319	-0.335	-0.307	-0.011	-0.294
	p	0.197	0.174	0.216	0.967	0.237
Log BCEA	*r*	0.664**	0.491**	0.662***	0.082	0.706***
	p	0.003	0.038	0.003	0.745	0.001
Log Eccentricity	*r*	0.113	0.403	0.081	-0.123	0.354
	p	0.667	0.109	0.759	0.639	0.163

$^{**}p < 0.05$, $^{***}p < 0.001$

Visual acuity had a significant positive correlation with the presence of drusen with overlying hyperreflective foci in the worse eye. Increased foci tended to have poorer visual acuity in the respective eye. Binocular visual acuity had significant positive correlation with drusen area, drusen with concave or convex shape and drusen with overlying hyperreflective foci in the worse eye. Binocular visual acuity was poorer with increasing incidence of the latter drusen characteristics in the worse eye. Fixation stability in the worse eye also had significant positive correlations with the same drusen characteristics as binocular acuity.

Table XXVI. Correlation of Visual Function with Drusen Reflectivity in the Worse Eye.

		High Reflectivity	Mid Reflectivity	Low Reflectivity
LogMAR VA	*r*	-0.020	0.092	0.122
	p	0.937	0.715	0.629
LogMAR VA OU	*r*	0.327	0.362	0.449
	p	0.185	0.139	0.062
Mars CS	*r*	-0.013	-0.127	-0.236
	p	0.958	0.615	0.346
Mars CS OU	*r*	-0.347	-0.284	-0.208
	p	0.145	0.254	0.407
Log BCEA	*r*	0.500**	0.462	0.438
	p	0.035	0.054	0.069
Log Eccentricity	*r*	0.098	0.263	0.238
	p	0.709	0.307	0.357

$^{**}p < 0.05$, $^{***}p < 0.001$

Fixation stability in the worse-eye had a significant positive correlation with drusen of high reflectivity in the same eye. As the presence of drusen with high reflectivity increased, the BCEA also increased, indicating more unstable fixation.

Table XXVII. Correlation with Visual Function and Drusen Content in the Worse Eye.

		Homogeneous	Nonhomogeneous	Core
LogMAR VA	*r*	0.034	0.030	0.506**
	p	0.893	0.905	0.032
LogMAR VA OU	*r*	0.195	0.433	0.720***
	p	0.438	0.072	<0.001
Mars CS	*r*	0.070	-0.056	-0.370
	p	0.783	0.825	0.131
Mars CS OU	*r*	-0.048	-0.248	-0.387
	p	0.851	0.321	0.113
Log BCEA	*r*	0.294	0.535**	0.750***
	p	0.237	0.022	<0.001
Log Eccentricity	*r*	0.074	0.027	0.358
	p	0.778	0.917	0.158

$^{**}p < 0.05$, $^{***}p < 0.001$

Visual acuity, binocular and worse-eye, had positive correlations with the presence of nonhomogeneous drusen with core in the worse eye. Fixation stability had significant positive correlations with nonhomogeneous drusen, with and without core.

Table XXVIII. Correlation of Neurominder/Neurotracker Parameters and Drusen Characteristics in the Better Eye.

		Drusen Area	Convex	Concave	Pointy	Foci
FO Direction (Log)	*r*	0.316	-0.017	0.380	-0.123	0.340
	p	0.188	0.945	0.109	0.615	0.154
SO Direction (Log)	*r*	0.409	-0.140	0.492**	0.359	0.353
	p	0.082	0.566	0.032	0.132	0.138
FO Orientation (Log)	*r*	0.199	-0.180	0.324	-0.169	0.250
	p	0.413	-0.128	0.176	0.489	0.301
SO Orientation (log)	*r*	0.387	0.172	0.470**	0.278	0.317
	p	0.102	0.482	0.042	0.249	0.186
3DMOT (Log)	*r*	-0.310	0.415	-0.444	0.150	-0.341
	p	0.197	0.077	0.057	0.539	0.153

$^{**}p < 0.05$, $^{***}p < 0.001$

Drusen considered concave in shape had positive correlations with SO direction and orientation. This indicates that individuals with more concave drusen had higher second order detection thresholds (poorer performance) on the Neurominder.

Table XXIX. Correlation of Neurominder/Neurotracker Parameters and Drusen Reflectivity in the Better Eye.

		High Reflectivity	Mid Reflectivity	Low Reflectivity
FO Direction (Log)	*r*	0.246	0.553**	-0.026
	p	0.310	0.014	0.917
SO Direction (Log)	*r*	0.469**	0.492**	-0.095
	p	0.043	0.032	0.699
FO Orientation (Log)	*r*	0.215	0.431	-0.183
	p	0.377	0.065	0.453
SO Orientation (Log)	*r*	0.467**	0.423	-0.093
	p	0.044	0.071	0.704
3DMOT (log)	*r*	-0.374	-0.446	0.164
	p	0.115	0.056	0.501

$^{**}p < 0.05$, $^{***}p < 0.001$

Drusen of high reflectivity had significant positive correlations with SO direction and orientation. The more drusen of high reflectivity an individual had in the better eye, the higher was the SO detection threshold (poorer performance) on the Neurominder. Drusen of mid reflectivity had significant positive correlations with FO and SO direction on the Neurominder. The more drusen of mid reflectivity an individual had in the better eye, the higher were the direction detection thresholds (poorer performance) on the Neurominder.

Table XXX. Correlation of Neurominder/Neurotracker Parameters and Drusen Content in the Better Eye.

		Homogeneous	Nonhomogeneous	Core
FO Direction (Log)	*r*	0.252	0.256	0.336
	p	0.299	0.290	0.159
SO Direction (Log)	*r*	0.066	0.360	0.374
	p	0.790	0.130	0.115
FO Orientation (Log)	*r*	0.057	0.180	0.165
	p	0.818	0.461	0.500
SO Orientation (Log)	*r*	-0.042	0.367	0.279
	p	0.865	0.122	0.247
3DMOT (log)	*r*	0.137	-0.348	-0.055
	p	0.576	0.145	0.823

$^{**}p < 0.05$, $^{***}p < 0.001$

There were no significant correlations with Neurominder or NeuroTracker parameters and drusen content in the better eye.

Table XXXI. Correlation of Neurominder/Neurotracker Parameters with Drusen Characteristics in the Worse Eye.

		Drusen Area	Convex	Concave	Pointy	Foci
FO Direction (Log)	*r*	0.141	0.108	0.156	-0.189	0.072
	p	0.578	0.668	0.536	0.453	0.775
SO Direction (Log)	*r*	0.355	0.374	0.345	-0.028	0.392
	p	0.148	0.126	0.161	0.911	0.107
FO Orientation (Log)	*r*	0.223	0.064	0.260	-0.271	0.511**
	p	0.373	0.801	0.297	0.277	0.030
SO Orientation (log)	*r*	0.304	0.244	0.303	0.013	0.373
	p	0.220	0.328	0.222	0.958	0.127
3DMOT (Log)	*r*	-0.366	-0.320	-0.380	0.212	-0.565**
	p	0.135	0.195	0.120	0.398	0.015

$^{**}p < 0.05$, $^{***}p < 0.001$

Drusen with overlying hyperreflective foci had a significant positive correlation with FO orientation on the Neurominder and a significant negative correlation with 3DMOT. The greater the incidence of foci, the higher the FO orientation detection threshold (poorer performance) on the Neurominder and the lower the speed threshold (poorer performance) on the 3DMOT.

Table XXXII. Correlation of Neurominder/Neurotracker Parameters with Drusen Reflectivity in the Worse Eye.

		High Reflectivity	Mid Reflectivity	Low Reflectivity
FO Direction (Log)	*r*	0.056	0.334	0.200
	p	0.826	0.175	0.848
SO Direction (Log)	*r*	0.173	0.225	0.447
	p	0.492	0.369	0.063
FO Orientation (Log)	*r*	0.023	0.373	0.395
	p	0.926	0.127	0.105
SO Orientation (Log)	*r*	0.224	0.284	0.428
	p	0.372	0.254	0.077
3DMOT (log)	*r*	-0.203	-0.518**	-0.485**
	p	0.420	0.028	0.042

**$p < 0.05$, ***$p < 0.001$

Drusen of mid and low reflectivity had significant, negative correlations with 3DMOT. The greater, the incidence of low or mid reflective drusen, the higher the speed threshold (poorer performance) on 3DMOT.

Table XXXIII. Correlation of Neurominder/Neurotracker Parameters and Drusen Content in the Worse Eye.

		Homogeneous	Nonhomogeneous	Core
FO Direction (Log)	*r*	-0.060	0.159	0.049
	p	0.812	0.528	0.848
SO Direction (Log)	*r*	0.266	0.267	0.447
	p	0.286	0.285	0.063
FO Orientation (Log)	*r*	-0.024	0.125	0.395
	p	0.924	0.621	0.105
SO Orientation (Log)	*r*	0.245	0.211	0.428
	p	0.327	0.401	0.077
3DMOT (log)	*r*	-0.128	-0.268	-0.485**
	p	0.613	0.282	0.042

$^{**}p < 0.05$, $^{***}p < 0.001$

Drusen with core has a significant, negative correlation with 3DMOT. The greater the incidence of drusen with core in the worse eye, the higher the speed threshold (poorer performance) on 3DMOT.

Interestingly, these were the only correlations between binocular vision and the drusen characteristics of the better eye.

In the worse eye, binocular visual acuity was significantly positively correlated with drusen area, drusen that were concave or convex in shape, nonhomogeneous drusen with core and drusen with overlying hyperreflective foci. This means that visual acuity got worse with the increasing incidence of these characteristics in the worse eye. Although one would expect binocular visual acuity to correlate with damage in the better eye, this finding does not disagree with the research that showed binocular vision is driven by the better eye (Tarita-Nistor et al., 2011). The current results show that although binocular summation is no longer possible, individuals still experience binocular vision on some level. Binocularity is still driven by the better eye, but degeneration occurring in the worse eye still has an effect on visual function.

In terms of drusen shape, those considered concave had the most significant correlations with visual function parameters. Convex drusen were positively correlated with fixation stability in the worse eye, while there were no correlations with drusen having a pointed shape. Presumably, this is due to the effect the shape has on the photoreceptor layer. Convex drusen have regular dome shapes, which elevate the photoreceptor layer, but appear not to disrupt it, while concave drusen have a more irregular shape, often making it hard to tell if the photoreceptor layer is still intact. If this is the case, then concave drusen would play a larger role in the function of the retina. Pointed drusen are relatively small compared to either concave or convex drusen and it has been suggested that these may represent a wrinkling of the outer limiting membrane rather than actual drusenoid deposits (Pieroni et al., 2006).

Drusen of high reflectivity were correlated with binocular contrast sensitivity and fixation stability in the worse eye, while mid reflective drusen were only correlated with fixation stability in the worse eye. An ultrahigh resolution OCT study conducted in 2006, matched drusen on OCT images to what was known from fundus photos at the time. It was suggested that drusen of mid reflectivity were soft drusen: extracellular debris, which is typically less reflective than the RPE. The same study suggested that high reflectivity

indicated migration of RPE cells (Pieroni et al., 2006). This migration could be happening in order to achieve better nutrient supply, or it could indicate migration in terms of cellular degeneration. If either theory is correct, then correlation with visual function would make sense and the results of this study would support the theories put forth by Pieroni et al. Following this line of thought, the mid reflective drusen would represent less mature drusen, having not been present long enough to cause permanent damage to the overlying RPE cells. This theory would explain why there were fewer correlations of visual function with drusen of mid reflectivity compared to drusen of high reflectivity. There were no correlations with drusen of low reflectivity, suggesting that it may not affect visual function. There is still some debate about what would correspond to the low reflectivity. Pieroni et al (2006) suggest that it could be an early sign of the development of wet AMD while another group has suggested these spots correspond to calcified sites on fundus photos (Khanifar et al., 2008).

The presence of nonhomogeneous drusen with core in the worse eye was significantly correlated with binocular visual acuity, visual acuity in the same eye and fixation stability in the same eye. This suggests that the most detrimental type of drusen content is that of the core, followed by nonhomogeneous drusen without core, which were also significantly correlated with fixation stability in the worse eye. There have been few studies discussing drusen content visible on images as OCT image analysis has only recently become possible. Khanifar et al. (2008) was the first to identify the drusen with core subtype. The core, seen on OCT, could correspond to the vesicular structures identified in histological studies (Anderson et al., 2004; L V Johnson et al., 2002). If this is the case, then perhaps the formation of a core requires the interaction between beta-amyloid and complement components. This would suggest that one of those components is missing from the drusen than do not contain a core. Given that the outer layer of the vesicle stains positive for complement components (Anderson et al., 2004; L V Johnson et al., 2002), nonhomogeneous drusen could primarily contain the inner layer of vesicles - beta-amyloid.

Retinal Structure – Cognitive Measures

This section of the study has shown the wealth of data that can be obtained from the study of retinal structure *in vivo* and different measures of perception and cognition. It seems that performances on cognitive measures are likely due to characteristics of retinal structure that affect visual function given the lack of correlations with the MoCA, rather than these characteristics being linked to cognitive impairment. Large amounts of data are not necessarily useful if connections cannot be drawn. The results here highlight the need to better refine the identification of drusen characteristics seen on OCT through machine learning. Given that OCT is a clinical staple, having software to identify characteristics that could be linked to functional or maybe even cognitive deficits would be an asset and much easier to incorporate into clinical practice than expensive new technology or persuading patients to take curcumin daily for a month to achieve sufficient dosage (Koronyo et al., 2017). It is also likely that these characteristics will have to be linked to histopathology before attempting to make connections to cognitive function

Conclusion

This study shows how retinal structure can impact visual function. The presence of drusen is related to poorer visual function in the more affected eye and to poorer binocular function. Despite the different functional abilities of eyes with AMD, some form of binocular vision is maintained. Binocular vision is driven by the better functioning eye but hindered by the structural damage in the worse eye.

The results of this study highlight the importance of visual input to perception and cognition. The AMD group, with impaired visual input, performed better on cognitive questionnaires where they were able to use stored experience and memory compared to psychophysical tests that depend more on visual cues. Perhaps in the cognitive evaluation of those with visual impairment, their ability to decipher meaning from limited visual input should be considered.

Chapter 7: General Discussion

The objective of this study was to learn more about the Age-related Macular Degeneration/cognitive impairment co-morbidity. This was the first study to examine the co-morbidity across the spectrum of genotype, retinal structure, and visual and cognitive function and one of a few to consider the same spectrum in a sample of individuals with AMD.

Traditional methods of evaluating retinal structure and visual and cognitive function were used, but newer technology such as scanning laser ophthalmoscope (SLO), optical coherence tomography (OCT) and psychophysical testing (Neurominder and 3DMOT) were incorporated with varying degrees of success. This study was successful in goals to replicate previous studies showing that the single nucleotide polymorphisms (SNPs) in complement factor H (CFH), rs1061170, and Age-related Maculopathy Susceptibility Gene 2 (ARMS2), rs10490924 occur more frequently in individuals with AMD than in not affected persons (Edwards et al., 2005; Fisher et al., 2005; Fritsche et al., 2008, 2014; Gatta et al., 2008; Hageman et al., 2005; Haines et al., 2005) and that more affected by AMD score positive for mild cognitive impairment (MCI) compared to the general population (AREDS Research Group, 2006; Boxerman et al., 2015; Duponsel et al., 2010; Wittich et al., 2014; Wong et al., 2002; Woo et al., 2012). Although this study did provide further insight on the AMD-MCI comorbidity and identified FADS1 rs174547 as possible contributor, it was less successful in showing a concrete relationship between the original SNPs of interest and MCI or drusen characteristics.

Genetic Findings

This participant-sample data in this study agrees with previous studies (Edwards et al., 2005; Fisher et al., 2005; Fritsche et al., 2008; Gatta et al., 2008; Hageman et al., 2005; Haines et al., 2005; R J Klein et al., 2005) showing that many individuals with AMD carry the SNPs CFHY402H and ARMS2A69S. In the case of each SNP, just over half of the AMD sample was identified as having at least one copy and individuals who were homozygous for these SNPs were far more common in the AMD group compared to

the control group. An unexpected finding was the frequency of the FADS1 SNP associated with AMD in the study sample. The minor allele frequency for this sample (AMD and control groups) was 0.85 which is higher than that reported for American (0.41) or European (0.66) populations (NIH, 2017). A large-scale population study would be required to confirm the theory of this being due to a Founder Effect. Given that the French-Canadian population from which this sample is derived is already a confirmed Founder population (Roy-Gagnon et al., 2011), the theory seems a likely explanation. FADS1 rs174547 has not been well characterized in the role it plays in AMD. Although identified in GWAS as a significant contributor to AMD (Neale et al., 2010), it has been overlooked for further study in favour of more influential SNPs such as CFHY402H and ARMS2A69S. The high frequency in the French-Canadian population would make this population ideal for studies aiming to better characterize the role of the FADS1 SNP in AMD.

Genetics and Visual Function

The results of this study support what is now common knowledge - the visual function of those with AMD is poorer compared to age-matched controls. The unique finding is that AMD risk SNPs CFHY402H and ARMS2A69S can be linked to poorer visual function in AMD, but also in individuals having normal vision. When participants with AMD and controls were pooled and analyzed according to the carrier status of these SNPs, it was found that carriers of ARMS2A69S performed worse on traditional measures of visual function such as visual acuity and contrast sensitivity, while carriers of CFHY402H had poorer performance on newer measures of visual function such as eccentric fixation. This shows that these SNPs contribute to AMD, but are not sufficient alone to cause the disease. This just further supports the multifactorial disease model of AMD.

There is still much speculation with respect to the function of the ARMS2 gene product (Kanda et al., 2007). It was identified in GWAS attempting to identify variants associated with AMD and was named accordingly. Over the last decade, many studies have attempted to characterize the function of the ARMS2 protein with little success.

Some of this is due to SNPs in ARMS2, also contributing to AMD, that prevent the gene from being transcribed into mRNA or cause the transcription of unstable mRNA that degrades before translation into protein (Fritsche et al., 2014). ARMS2A69S is a SNP that occurs in the coding region of the gene that does not affect its transcription. Early localization studies established that ARMS2 was expressed in the human retina and localized its expression to photoreceptors (Fritsche et al., 2008). Subsequent studies refined its localization to the inner segments of both rods and cones, specifically, the mitochondria-enriched ellipsoid region of photoreceptor inner segments (Fritsche et al., 2014). This led to the hypothesis of a role in mitochondrial homeostasis. Mitochondrial-associated diseases are increasingly recognized as due to disturbances in cellular energy supply, generation of reactive oxygen species and/or initiation of apoptosis (Lin & Beal, 2006), all of which have been implicated as contributing to AMD development (Garcia-Escudero et al., 2013; Kaarniranta et al., 2011; Katta et al., 2009; Sivak, 2013).

The mitochondrial localization of ARMS2 and the association of the A69S variant with oxidative stress could have dire consequences for retinal photoreceptors. Increased levels of oxidative stress, reactive oxygen species or lack of cellular energy could be causing photoreceptor mitochondria to trigger apoptosis and subsequent cell death. This would explain the association of the presence of ARMS2A69S with poor contrast sensitivity and visual acuity given these parameters are dependent on the integrity of the photoreceptors themselves. Further, this is supported by the dose-dependent nature of the SNP. Those heterozygous for ARMS2A69S have one wild type copy of ARMS2 and while non-carriers would have two wild type copies. Photoreceptors expressing the wild type ARMS2 would not have the same fate as those expressing the risk variant, explaining the better performance of non-carriers and then heterozygotes compared to homozygotes on contrast sensitivity and visual acuity.

Carriers of CFHY402H have poorer performance on visual function in terms of having more eccentric fixation compared to non-carriers. Oddly, carriers did not display poorer visual acuity or fixation stability compared to non-carriers. This disagrees with previous research showing that greater eccentricity correlates with visual acuity and poorer fixation stability (Provis et al., 2013). This trend was only seen in carriers versus

non-carriers of CFHY402H as the expected correlation occurred in the sample as a whole. This is possibly explained by CFHY402H being linked to greater incidence of drusen and not the function of retinal cells. This theory is supported by the current study showing that carriers of this SNP have greater drusen area compared to non-carriers. Drusen formation, particularly in the central retina would disrupt the architecture of the retina, forcing fixation outwards. Given the link between the CFH SNP and the early stages of AMD (Dietzel et al., 2014), carriers would likely have time to adapt to the change in eccentricity, improving their visual acuity and fixation stability.

It appears that ARMS2A69S is linked to photoreceptor dysfunction and CFHY402H is linked to disruption of retinal architecture through drusen. Those carrying both SNPs would have compounding factors of photoreceptor dysfunction and disruption of retinal architecture, supporting the increased incidence of AMD among carriers of both SNPs (Gorin, 2012; J M Seddon, Reynolds, Yu, Daly, & Rosner, 2011; Seddon, Reynolds, Yu, & Rosner, 2013; Seddon et al., 2009). It remains to be seen, however, why some individuals who carry these SNPs do not develop AMD. Multifactorial disease models suggest environmental and lifestyle factors could be deciding factors. Perhaps carriers of ARMS2A69S who are vigilant about wearing sunglasses and/or have brown eyes have made a difference in protecting their retinas from oxidative stress caused by the sun enough to prevent the development of AMD. Meanwhile, those who do not wear sunglasses and/or have less pigment in their irises are more exposed to sun damage, and have the added dysfunction of ARMS2A69S leading to AMD. Conversely, non-smoking carriers of CFHY402H would have less systemic inflammation, creating less demand on CFH and the alternative complement system, enabling the retina to better clear debris. Meanwhile, carriers who are also smokers would have increased demands on the alternative complement while would likely not be able to compensate along with the CFH SNP.

Genetics and Retinal Structure

This research is the second attempt to connect AMD SNPs to drusen characteristics via OCT. To date, there has been little attention given to AMD risk

variants and drusen accumulation (Boon et al., 2008; Munch et al., 2010; Yu et al., 2011), let alone any connection these that variants may have to OCT-derived drusen measurements that can be monitored *in vivo*. A 2015 study reports an association between the CFH rs12038333 risk SNP and drusen area and RPE atrophy through univariate analyses (Ramana et al., 2015). Although rs12038333 differs from the CFHY402H SNP discussed in the present study, both studies link mutations in the CFH gene with drusen suggesting that drusen are linked to dysfunction of the CFH gene product rather than a specific SNP. The present study, although using a much smaller sample size, reports significant correlations of CFHY402H with drusen area and percentage of the macula covered by geographic atrophy (GA) and goes a step further, linking the CFHY402H SNP with drusen of mid reflectivity.

Findings are in agreement with Ramana *et al*. (2015) in terms of ARMS2A69. The same SNP was used in both studies and neither found a significant correlation with drusen area or percentage of the macular affected by GA. In examining more extensive OCT-derived drusen characteristics, this study found significant correlations with drusen of mid reflectivity and drusen of homogeneous content.

Genetics and Cognitive Function

No significant differences in cognitive score on the MMSE or MoCA questionnaires were found between carriers and non-carriers of the CFHY402H and ARMS2A69S SNPs. In this sample, it appears that these SNPs may not play a role in cognitive impairment, but as mentioned previously, a larger sample size would provide more interpretable. Conversely, the results show that the FADS1 SNP may have a role in cognitive impairment given that all those scoring positive for MCI according to the MoCA were carriers of the SNP. It was also shown that individuals carrying two copies of FADS1 rs174547 had lower cognitive scores compared to those with only one copy or none at all. Although the discovery of the potential FADS1 rs174547 Founder Effect in the Quebec population is interesting and has implications for future study of the epidemiology of AMD, it is problematic in attempting to link the SNP to an AMD-MCI co-morbidity is problematic. To confirm a link between the FADS1 SNP and MCI, the

study should be repeated, but with a larger sample with a carrier versus non-carrier distribution.

There were no significant differences between threshold responses on the Neurominder or 3DMOT with respect to carriers versus non-carriers of the SNPs of interest, including FADS1. When zygosity was considered, the Neurominder showed a significant difference between carrier status of ARMS2A69S and threshold for first order (FO) stimuli. Those with two copies of the ARMS2 SNP had a higher threshold elevation compared to heterozygotes and non-carriers, indicating poorer performance. Research on object attributes has shown that FO attributes are processed in V1 and higher order functions, like visual working memory, are not required for rectification (Faubert, 2002; Habak & Faubert, 2000). Current views surmise that the neuropathology of AD begins in the limbic system and prelimbic cortices before extending into higher order posterior association areas, then to lower-order association area like V1 (Albers et al., 2015; Braak & Braak, 1995; Lewis, Campbell, Terry, & Morrison, 1987) Based on this view, it would make more sense for the poorer Neurominder scores to be on the detection of second order (SO) stimuli rather than FO given the requirement of visual working memory and higher order association cortices to rectify SO attributes. This is supported by other studies on AD and object attributes that found deficits in the perception of motion, a SO attribute, compared to individuals without symptoms of dementia (Albers et al., 2015; Gilmore et al., 1994; Rizzo & Nawrot, 1998). It was thought that this could be due to selective damage to the magnocellular pathway, the retino-cortical pathway that connects the retina to areas 17 and 18 of the primary visual cortex. Here, information is then further projected to the middle temporal cortex, which plays a role in the perception and integration of motion-related information (Chang et al., 2014; Hof & Morrison, 1990). This is supported by significant cellular loss in specific layers of area 17 and 18 that project to motion-processing areas (Hof & Morrison, 1990).

It would seem that if carriers of ARMS2A69S demonstrated deficits in detection of FO stimuli, they would also display deficits in the detection of SO stimuli or, at least, obtain poorer scores on SO direction given the implications of cellular loss in the magnocellular pathway (Chang et al., 2014; Gilmore, Groth, & Thomas, 2006; Hof &

Morrison, 1990; Rizzo & Nawrot, 1998). This was not the case. The deficits in detection of solely FO stimuli lead to the hypothesis that these results are not due to top-down, cortical deficits alone. It could be possible that those with the ARMS2 SNP are affected by the top-down neuropathology that starts in the limbic system in addition to bottom-up neuropathology that begins in the retina and visual cortices. Evidence from histopathological studies in transgenic mice (Koronyo-Hamaoui et al., 2011) and in humans (McKee et al., 2006) have shown that posterior cortical visual areas can be substantially affected by neuropathology prior to the onset of the symptoms of dementia. Having top-down and bottom-up neuropathologies could explain the higher prevalence of MCI among those with AMD compared to the general population. However, a second option and the more likely of the two, is that the FO deficits are simply due to the retinal pathology of AMD and the visual cortex is not affected. Of course, diminished visual input from the retina due to either aging or visual pathology has been considered. Some studies have gone on to show that the visual information received from the retina can be improved, if not recovered, by increasing the contrast of the visual stimuli (Cronin-Golomb et al., 1991; Gilmore et al., 2006). The current study would support this due to results showing carriers of ARMS2A69S to have significantly poorer contrast sensitivity compared to non-carriers. To rule out the first hypothesis, imaging studies beyond the scope of this dissertation would be required to identify any structural abnormalities in the visual cortex.

The most obvious limitation in the present study is the sample size. Recruitment based on genotype is not yet possible and leaving it to chance is costly. When studying the association between a SNP and a complex disease, many factors beyond that of effect size and alpha level must be taken into consideration. Some of these include disease prevalence, linkage disequilibrium, allele frequency and inheritance model. To evaluate a single SNP, it is recommended to have 248 cases and the equivalent number of control subjects. This is based on an odds ratio of 2, a disease prevalence of 5%, a minor allele frequency of 5%, complete linkage disequilibrium, a 1:1 case-control ratio and a 5% error rate in allelic testing (Hong & Park, 2012). In addition, this study investigated the association between AMD SNPs and the AMD-MCI co-morbidity. The higher prevalence

of MCI among those with AMD has only recently come to light and to date, there are few studies examining genetic contributions (Logue et al., 2014; Lukiw, Surjyadipta, et al., 2012; Proitsi et al., 2012; Williams et al., 2015). Due to this state of knowledge, many of the factors involved in determining a reliable sample size have not yet been established.

The Co-morbidity: AMD versus Controls

The results of this study agree with others reporting a higher prevalence of MCI among those with AMD compared to that in the general population. This study found that approximately 38% of individuals with AMD scored positive for cognitive impairment according to the MoCA. This study had a small sample size, but the rates are between 30 and 50%, similar to other studies using the MoCA (Duponsel et al., 2010; Wittich et al., 2014) and higher than those of studies using other measures of cognitive status (Klaver et al., 1999; Whitson et al., 2010; Wong, Iu, Koizumi, & Lai, 2012). This prevalence is higher than that of MCI or AD in general population, which was reported to be 3-19% (Gauthier et al., 2006) and 5-7% (World Health Organization, 2012a) of individuals over the age of 60, respectively.

Although the AMD and control groups in this study did not differ significantly on their average MoCA scores, they did differ on which subscales they found most difficult. Individuals from the control group who scored positive for MCI had difficulty with delayed recall, or memory. Individuals with AMD who scored positive for MCI had difficulty with delayed recall in addition to the abstraction and orientation subscales. Not all diagnoses of MCI convert to AD or another form of dementia. Prospective research has shown that the cases of MCI most likely to convert to AD are those that display memory deficits in addition to impairment other cognitive domains (Summers & Saunders, 2012). The results herein show that persons with AMD and MCI may be more likely to develop AD compared to controls with MCI. Of course, this should be confirmed with prospective studies in an AMD population and more rigorous testing of distinct cognitive domains, but it has strong implication for low vision rehabilitation. Low vision rehabilitation depends largely on training and one's ability to understand and remember instructions. Research in a low vision rehabilitation setting has reported a

higher risk of functional disability and poorer quality of life for individuals with vision impairment and cognitive deficits compared to those with vision impairment alone (Whitson et al., 2007). When the traditional low vision rehabilitation program was augmented to accommodate cognitive decline, improvements in both visual function and cognitive abilities were seen (Whitson et al., 2013). Rehabilitation programs like this are not yet common practice, but could have a significant impact on the effectiveness of services for individuals with a vision impairment/cognitive impairment co-morbidity.

Conclusion

In the past, disease was identified solely by dysfunction. AMD and AD were simply age-related vision loss and dementia respectively. As science advanced, these functional outcomes were traced back to changes in the affected individual's organs or tissues, leading to the discovery that a disease mechanism was already well underway before symptoms began. In the 1800s, ophthalmoscopy identified yellowish spots on the retina of those with central vision loss and in 1906 histological study of post-mortem brain tissue found plaques in the brains of individuals with Alzheimer's disease (Alzheimer et al., 1995). More recently, science has reached the other end of the spectrum - genotype. This had led to the understanding that genes contain the blueprint for tissue architecture and this architecture is what determines function. Based on this concept, disease prediction models are beginning to be constructed and waiting for the functional deficits of a disease to become apparent is no longer necessary. By that time, the process is likely too advanced to reverse. With the sequencing of the human genome, genotype can be determined at any point during the lifespan and provided that enough is known about the genotype-structure-function relationship, disease outcomes can be predicted. Science is evolving with the ultimate goal of being able to prevent disease by knowing the genotype right from the start. In some instances, science is on the brink of success with gene replacement therapies as in the case Leber's Congenital Amourosis (Coussa, Lopez Solache, & Koenekoop, 2017) while, in other cases, it is still a struggle to achieve a definitive diagnosis before death.

Some disease models are simple, like that of Stargardt disease, the junvenile form of AMD. Inheriting two mutated copies of ABCA4, a gene encoding a retina-specific protein, leads to the disease. The protein is important in the clearance of toxins produced during photoreceptor outer-segment recycling. These toxins are made during photobleaching and their accumulation leads to photoreceptor degeneration (Allikmets, Singh, et al., 1997). Here, a mutation in the gene disrupts tissue architecture, which ultimately alters function. This creates many different domains in which to study a disease, each requiring experts in genetics, molecular biology, therapeutics, rehabilitation, etc. Not all diseases are as straightforward as Stargardt disease. AMD and AD are much more complex having gene interactions and environmental factors to consider, creating even more perspectives from which to study the disease mechanism. In terms of complex disease, the best way to reach that ultimate goal of prevention is to increase communication across the spectrum.

The goal of this research was exactly that - to explore the relationship across the spectrum of genotype, structure and function in AMD and cognitive impairment. In doing so, FADS1 rs174547 has been identified as a potential genetic target in this co-morbidity. The French-Canadian population has also been identified as a prime population to better understand the role of the FADS1 SNP in the AMD disease mechanism due to the high frequency with which it occurs in this population.

The known AMD SNPs CFHY402H and ARMS2A69S appear not to play a major role in this comorbidity. Nonetheless, this research has contributed to a better understanding of their role in AMD pathogenesis. The CFH SNP was linked to more eccentric fixation leading to the hypothesis that it plays a role in the disruption of retinal architecture. This was further supported by correlations with drusen area and greater amounts of geographic atrophy. Conversely, the ARMS2 SNP was linked to deficits in visual acuity and contrast sensitivity, visual functions localized to the photoreceptors themselves. In terms of retinal structure ARMS2A69S was linked to drusen of a homogenous nature, leading again to the hypothesis that the SNP contributes to AMD through the loss of photoreceptor integrity.

In addition to genetic findings, this study adds to the growing body of literature reporting a high prevalence of cognitive impairment in AMD. This research also supports previous studies demonstrating that the MoCA is more effective in detecting mild forms of cognitive impairment compared to the MMSE. Additionally, the cognitive domains screened by the MoCA show a difference between individuals with AMD and age-matched controls. Those with AMD scoring positive for MCI have difficulty in other cognitive domains in addition to memory, indicating a higher risk of conversion to Alzheimer's Disease.

By establishing a relationship between genotype, structure and function, both diseases will be better understood making it possible for genotype alone to become a predictive measure. Genotype can be determined early in life. Given that some risk factors for these diseases are modifiable, those carrying risk variants might be able to take preventative measures, decreasing their chances of developing AMD, MCI or AD. Finding a genetic link between two such prevalent diseases would provide a target for drug development. With the emerging fields of pharmacogenetics and personalized medicine, it is becoming more important to understand how genetics can influence the onset, progression and treatment-response of disease. In addition, rehabilitation specialists would be able to personalize services and anticipate cognitive decline for those with the high-risk genes. This, in turn, will make the rehabilitation service delivery process more efficient and effective.

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Appendix A: Consent forms

Consent Form

Visual Impairment/Cognitive Impairment Co-morbidity; Examining the Genotype-Structure-Function Relationship

(Primary Participants, genotyped)

Researcher	**Caitlin Murphy, M. Sc.** Ph.D. student School of optometry, University of Montreal.
Supervisors	**Olga Overbury, Ph. D.** Professor School of optometry, University of Montreal. **Jocelyn Faubert, Ph. D.** Professor School of optometry, University of Montreal

Funding: none.

Preamble

We are inviting you to take part in a genetic study. However, before you decide and sign the Information and Consent Document, take the time to read, understand and carefully think about the following information.

This Information and Consent Document may contain information or words that you do not understand. You should ask the researcher in charge of the study or members of the study staff to answer your questions and explain any word or statement that you do not understand.

Nature and Objectives of Study

The number of Canadian seniors affected by Age-related Macular Degeneration (AMD) and early cognitive changes is on the rise. While AMD affects abilities such as reading and driving, cognitive changes

can reduce one's ability to understand and to communicate. Thus, both conditions severely affect quality of life, independence and social participation. Currently, the causes of AMD and cognitive impairment are not well understood and there are few, if any, effective treatments. As life expectancy increases and our population ages, more cases will develop. In a recent study, one of five older adults receiving low vision rehabilitation for macular disease scored positive for cognitive impairment. The high occurrence of these occurring together suggests that visual and cognitive impairments may be related.

The goal of this study is to explore the relationship among genotype (the variation in your genes), the structure of your eye and your visual function. By establishing a relationship between genotype, structure and function, both conditions will be better understood making it possible for genotype alone to become a predictive measure. Genotype can be determined early in life. Those carrying risk genes can take preventative measures earlier in life, decreasing their chances of developing AMD or cognitive impairment.

Participation

You are being recruited for this study because you have been diagnosed with AMD and recently volunteered to participate in a genotyping study conducted at the Montreal Retina Institute. If you decide to participate, information from Dr. Chen's study and your current visual function will be used. The testing will occur over two sessions. Test sessions will occur at the School of Optometry at the University of Montreal and will also last approximately 45 minutes. There will be two tests session in total and they will be scheduled at your convenience.

Study Procedure

First session (45 minutes)

1. It will begin with a short questionnaire that collects information such as your age, language, heritage, etc.
2. Visual function testing will also take place during the first session. This collection of tests will measure visual acuity, contrast sensitivity, colour vision and reading acuity
3. Two eye drops per eye (2.5% phenylephrine® and 1% tropicamide®) will be administered in order to dilate your pupils. This allows us to take better pictures of the back of your eyes.

4. While waiting for your pupils to dilate, you will then respond to two questionnaires; the Montreal Cognitive Assessment (MoCA) and the Mini-Mental State Exam (MMSE) which will be administered to measure cognitive status. These questionnaires are typically used clinically to detect or measure the severity of cognitive impairment and monitor cognitive changes over time. They cover a number of categories including orientation to time and place, repeating lists of words, simple math, language use and understanding, and basic motor skills.
5. A colour fundus camera will be used to take pictures of your eyes.

Second session (45 minutes)

1. It will include use of a device called ophthalmoscope (OCT/SLO). This device, commonly used in ophthalmology exams, will be used to:
   a. Observe and evaluate the layers of your retina (the back of your eye).
   b. View the retinal surface in real time.
   c. Measure the response of your eye to small lights.
   d. Determine the borders of any scarring resulting from your AMD can be located.
2. Lastly, the NeuroMinder and NeuroTracker, both by Cognisens, are computer programs that measure changes in visual perception (how you see things). The NeuroMinder involves viewing stripes of varying contrast and determining the direction of motion (up, down, left or right). The NeuroTracker involves tracking the movement of bouncing balls in a 3D environment. This will be achieved by wearing 3D glasses

Study Risks

All tests are non-invasive but, do require use of your eyes. There is a risk of your eyes becoming tired and teary from testing.

Dilation drops are commonly used by eye doctors during routine eye exams. They are required for taking pictures of your retinas. Side effects to the drops are possible. Minor irritation may be felt when first putting in the drops. This will last approximately 10 seconds. When dilation is maximal, your pupil will appear larger and near vision will be blurry. Reading and any activity up close will be difficult for a period of up to six hours after first putting in the drops. Your pupil will appear larger for approximately the same amount of time. It is possible that

you will be more sensitive to light following dilation of the eye. It is therefore recommended to wear sunglasses outside when dilated.

You should not drive on the day of the testing because your pupils may be dilated. Driving is not recommended when dilated because dilation can distort vision temporarily. If you are coming by car, then you need to have someone drive you. It is not necessary to be accompanied if you are taking the subway, bus or any other form of transportation where you will not put others at risk while your vision is distorted.

Disadvantages Associated with this Study

This study occurs in two sessions at the University of Montreal. Due to pupil dilation, you will be asked to either use public transportation or have someone drive with you to the testing session.

Advantages

This research is on-going and will take many years, it is unlikely that you will get any direct benefit from taking part in this study. This research may lead to better diagnosis and treatment in future for patients who have the same or a similar condition as you.

Compensation

This study is strictly voluntary. You will not be compensated for your time.

Dissemination of Results

Reports about your DNA testing can be made available to you. A research assistant will go over the report with you. Be aware that this information may not affect only you, but relatives that share your genes as well. Laboratory report interpretation and genetic counseling services can be made available to you.

Evaluation of the overall results will only be performed as a group and not by individual patient. The medical implications of the results of this testing, if any, will only be known after many studies like this one are done. If requested, a summary of the findings from this research can be given over the phone.

If the results of the study are published, your name will not be used and no information that discloses your identity will be released or

published.

Voluntary Participation and the Right to Withdraw

You may choose whether you would like to take part in this study. If you choose to take part now, you can change your mind later and stop at any time and for any reason with a simple verbal request. Your future medical care and your relationship with your doctor and or other people involved in your care will not change in any way.

Confidentiality

All the information collected about you during the study will remain confidential. To protect your identity and name, identifying information will be replaced with a code (numbers and/or letters), the link between the code and your identity will be held by the researcher in charge of the study. Only the researcher responsible for the study (Caitlin Murphy) and Olga Overbury will have direct access to this information. Your results will be kept secure within the Low Vision Lab at the school of optometry and will be destroyed 10 years after the end of this study. In the case that these results are published, your name and identity will not be revealed. No information that discloses your identity will be allowed to leave the institution.

Evaluation of the overall results will only be performed as a group and not by individual patient. The medical implications of the results of this testing, if any, will only be known after many studies like this one are done.

Contact

If you have questions about the study or if you feel you have a problem related to taking part in the study, you can communicate with the researcher in charge of the study.

For any information of an ethical nature regarding your participation in this study, you can contact the research ethics advisor of the University of Montreal's health research ethics board (CERES).

For more information on your rights as participants in the study, we invite you to consult the University of Montreal participants' webpage at: http://recherche.umontreal.ca/participants (French only).

Any complaints concerning this research can be addressed to the ombudsman of the University of Montreal. It is possible to contact the ombudsman toll-free. The ombudsman is fluent in French and English, and will take your calls between 9:00 AM and 5:00 PM.

Consent

I have read and reviewed the Information and Consent Document and the study was explained to me. My questions were answered to my satisfaction. I was given the time to think about whether I want to take part in this study.

I agree to take part in this study according to the conditions set in this Information and Consent Document. A dated and signed copy of this Information and Consent Document will be given to me.

First and last name (Participant)	Signature
	Date :

As a member of the research team, I explained the research project to _____ (name of participant). I answered his/her questions and informed him/her of their rights. The latter told me that he/she agreed to participate in this research project. For my part, I pledge to conduct the research project as described and discussed. Consent form administered and explained in person by:

First and last name (Researcher)	Signature
	Date :

Consent Form

Visual Impairment/Cognitive Impairment Co-morbidity; Examining the Genotype-Structure-Function Relationship

(consent form control group, no AMD)

Researcher	**Caitlin Murphy, M. Sc.** Ph.D. student School of optometry, University of Montreal.
Supervisors	**Olga Overbury, Ph. D.** Professor School of optometry, University of Montreal **Jocelyn Faubert, Ph. D.** Professor School of optometry, University of Montreal

Funding: none.

Preamble

We are inviting you to take part in a genetic study. You are being recruited for this study because you do not have AMD and have no reported family history of AMD. However, before you decide and sign the Information and Consent Document, take the time to read, understand and carefully think about the following information.

This Information and Consent Document may contain information or words that you do not understand. You should ask the researcher in charge of the study or members of the study staff to answer your questions and explain any word or statement that you do not understand.

Nature and Objectives of Study

The number of Canadian seniors affected by Age-related Macular Degeneration (AMD) and early cognitive changes is on the rise. While

AMD affects abilities such as reading and driving, cognitive changes can reduce one's ability to understand and to communicate. Thus, both conditions severely affect quality of life, independence and social participation. Currently, the causes of AMD and cognitive impairment are not well understood and there are few, if any, effective treatments. As life expectancy increases and our population ages, more cases will develop. In a recent study, one of five older adults receiving low vision rehabilitation for macular disease scored positive for cognitive impairment. The high occurrence of these occurring together suggests that visual and cognitive impairments may be related.

The goal of this study is to explore the relationship among genotype (the variation in your genes), the structure of your eye and your visual function. By establishing a relationship between genotype, structure and function, both conditions will be better understood making it possible for genotype alone to become a predictive measure. Genotype can be determined early in life. Those carrying risk genes can take preventative measures earlier in life, decreasing their chances of developing AMD or cognitive impairment.

Participation

You are being recruited for this study because you have normal vision (have not been diagnosed with AMD). If you decide to participate, information about your current visual function will be measured and a DNA sample for genotyping will be obtained. The testing will occur over two sessions. Test sessions will occur at the School of Optometry at the University of Montreal and will also last approximately 45 minutes. There will be two tests session in total and they will be scheduled at your convenience.

Study Procedure

First session (45 minutes)

1. It will begin with a short questionnaire that collects information such as your age, language, heritage, etc.
2. Visual function testing will also take place during the first session. This collection of tests will measure visual acuity, contrast sensitivity, colour vision and reading acuity
3. Two eye drops per eye (2.5% phenylephrine® and 1% Tropicamide® will be administered in order to dilate your pupils. This allows us to take better pictures of the back of your eyes.

4. While waiting for your pupils to dilate, you will respond to two questionnaires; the Montreal Cognitive Assessment (MoCA) and the Mini-Mental State Exam (MMSE) which will be administered to measure cognitive status. These questionnaires are typically used clinically to detect or measure the severity of cognitive impairment and monitor cognitive changes over time. They cover a number of categories including orientation to time and place, repeating lists of words, simple math, language use and understanding, and basic motor skills.
5. A DNA sample will be collected by rubbing a swab along the inside of your cheek. Your sample will be sent to Asper BioTech who will identify any AMD risk genes you may have.
6. A colour fundus camera will be used to take pictures of your eyes.

Second session (45 minutes)

1. It will include use of a device called ophthalmoscope (OCT/SLO). This device, commonly used in ophthalmology exams, will be used to:
   a. Observe and evaluate the layers of your retina (the back of your eye).
   b. View the retinal surface in real time.
   c. Measure the response of your eye to small lights.
   d. Determine the borders of any scarring resulting from your AMD can be located.
2. Lastly, the NeuroMinder and NeuroTracker, both by Cognisens, are computer programs that measure changes in visual perception (how you see things). The NeuroMinder involves viewing stripes of varying contrast and determining the direction of motion (up, down, left or right). The NeuroTracker involves tracking the movement of bouncing balls in a 3D environment. This will be achieved by wearing 3D glasses.

Study Risks

All tests are non-invasive but, do require use of your eyes. There is a risk of your eyes becoming tired and teary from testing.

Dilation drops are commonly used by eye doctors during routine eye exams. They are required for taking pictures of your retinas. Side effects to the drops are possible. Minor irritation may be felt when first putting in the drops. This will last approximately 10 seconds. When dilation is maximal, your pupil will appear larger and near vision will be blurry. Reading and any activity up close will be difficult for a period of up to six hours after first putting in the drops. Your pupil will appear larger for approximately the same amount of time. It is possible that you will be more sensitive to light following dilation of the eye. It is therefore recommended to wear sunglasses outside when dilated.

You should not drive on the day of the testing because your pupils may be dilated. Driving is not recommended when dilated because dilation can distort vision temporarily. If you are coming by car, then you need to have someone drive you. It is not necessary to be accompanied if you are taking the subway, bus or any other form of transportation where you will not put others at risk while your vision is distorted.

When you give a DNA sample for research, you share genetic information, not only about yourself, but also biological (blood) relatives who share your genes or DNA. There is a risk that information from genetic research could possibly be tied to you. The potential re-identification of information (eg, by an employer or insurer) may lead to loss of privacy and possible future discrimination in employment or insurance, against you or your biological relatives. You should be aware that genetic information cannot be protected against disclosure by court order.

Please indicate your choice regarding knowledge of your AMD genotype.

☐ I understand the risks and want the results of my genetic test. I understand that AMD is a complex disease influenced by genes and the environment. Asper BioTech will determine which gene variants I carry and calculate the risk of development of AMD. Carrying AMD variants does not mean I will get the disease for certain.

☐ I do not want the results of my genetic test.

Disadvantages Associated with this Study

This study occurs in two sessions at the University of Montreal. Due to pupil dilation, you will be asked to either use public transportation or have someone drive with you to the testing session.

Advantages

This research is on-going and will take many years, it is unlikely that you will get any direct benefit from taking part in this study. This research may lead to better diagnosis and treatment in future for patients who have the same or a similar condition as you.

Compensation

This study is strictly voluntary. You will not be compensated for your time.

Dissemination of Results

Reports about your DNA testing can be made available to you. Asper BioTecjh provides a report in simple terms that explain the genetic markers you have and your risk of developing AMD. A research assistant will go over this report with you. Be aware that this information may not affect only you, but relatives that share your genes as well. Laboratory report interpretation and genetic counseling services can be made available through Asper Biotech.

Evaluation of the overall results will only be performed as a group and not by individual patient. The medical implications of the results of this testing, if any, will only be known after many studies like this one are done. If requested, a summary of the findings from this research can be given over the phone.

If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published.

Voluntary Participation and the Right to Withdraw

You may choose whether you would like to take part in this study. If you choose to take part now, you can change your mind later and stop at any time and for any reason. Your future medical care and your relationship with your doctor and or other people involved in your care will not change in any way.

Confidentiality

All the information collected about you during the study will remain confidential within the limits of the Law. To protect the your identity and name, identifying information will be replaced with a code (numbers and/or letters), the link between the code and your identity will be held by the researcher in charge of the study. Only the researcher responsible for the study (Caitlin Murphy) and Dr. Overbury will have direct access to this information. Your results will be kept secure within the Low Vision Lab at the school of optometry and will be destroyed after 10 years. In the case that these results are published, your name and identity will not be revealed. No information that discloses your identity will be allowed to leave the institution.

Contact

If you have questions about the study or if you feel you have a problem related to taking part in the study, you can communicate with the researcher in charge of the study.

For any information of an ethical nature regarding your participation in this study, you can contact the coordinator of the University of Montreal's health research ethics board (CERES).

For more information on your rights as participants in the study, we invite you to consult the University of Montreal participants' webpage at: http://recherche.umontreal.ca/participants.

Any complaints concerning this research can be addressed to the ombudsman of the University of Montreal. It is possible to contact the ombudsman toll-free. The ombudsman is fluent in French and English, and will take your calls between 9:00 AM and 5:00 PM.

Consent

A copy of the Information and Consent Document will be placed in my medical file. Therefore, I understand that they are available to any person or organization that has access to my medical file.

I have read and reviewed the Information and Consent Document and the study was explained to me. My questions were answered to my satisfaction. I was given the time to think about whether I want to take part in this study.

I agree to take part in this study according to the conditions set in this Information and Consent Document. A dated and signed copy of this Information and Consent Document will be given to me.

First and last name (Participant)	Signature
	Date :

As a member of the research team, I explained the research project to _____ (name of participant). I answered his/her questions and informed him/her of their rights. The latter told me that he/she agreed to participate in this research project. For my part, I pledge to conduct the research project as described and discussed. Consent form administered and explained in person by:

First and last name (Researcher)	Signature
	Date :

Appendix B: Measures of Visual Function

ETDRS Visual Acuity Assessment

DISTANCE VISUAL ACUITY:

Correction Used # _____

Letter Size	OD Chart 1	OS Chart 2	OU Chart R	Letters Correct
40 M	NCKZO	DSRKN	HVZDS	5
32 M	RHSDK	CKZOH	NCVKD	10
25 M	DOVHR	ONRKD	CZSHN	15
20 M	CZRHS	KZVDC	ONVSR	20
16 M	ONHRC	VSHZO	KDNRO	25
12 M	DKSNV	HDKCR	ZKCSV	30
10 M	ZSOKN	CSRHN	DVOHC	35
8 M	CKDNR	SVZDK	OHVCK	40
6 M	SRZKD	NCVOZ	HZCKO	45
5 M	HZOVC	RHSDV	NCKHD	50
4 M	NVDOK	SNROH	ZHCSR	55
3 M	VHCNO	ODHKR	SZRDN	60
2.5M	SVHCZ	ZKCSN	HCDRO	65
2 M	OZDVK	CRHDV	RDOSN	70

	OD	OS	OU
Chart Distance (Meters)			
Letter size of last line read (3 / 5 correct)			
Letters Correct			
ACUITY	20 /	20 /	20 /

PERFORMANCE DURING BINOCULAR ACUITY (OU):

READING SPEED: ☐ Slow ☐ Average ☐ Fast

☐ Head turn(Right / Left / Up / Down)

☐ Consistent omissions of a quadrant... (Right / Left / Upper / Lower)

☐ Other: _____

Chart Distance

No. of Letters Correct	1 meter (20/x)	2 meters (20/x)
1	962	481
2	919	459
3	877	439
4	838	419
5	800	400
6	764	382
7	730	365
8	697	348
9	665	333
10	635	318
11	607	303
12	580	290
13	553	277
14	529	264
15	505	252
16	482	241
17	460	230
18	440	220
19	420	210
20	401	200
21	383	191
22	366	183
23	349	175
24	333	167
25	318	159
26	304	152
27	290	145
28	277	139
29	265	132
30	253	126
31	242	121
32	231	115
33	220	110
34	210	105
35	201	100
36	192	96
37	183	92
38	175	88
39	167	84
40	160	80
41	152	76
42	146	73
43	139	70
44	133	66
45	127	63
46	121	61
47	116	58
48	110	55
49	105	53
50	101	50
51	96	48
52	92	46
53	88	44
54	84	42
55	80	40
56	76	38
57	73	36
58	70	35
59	67	33
60	64	32
61	61	30
62	58	29
63	55	28
64	53	26
65	50	25
66	48	24
67	46	23
68	44	22
69	42	21
70	40	20

Mars Letter Contrast Sensitivity Test

The Mars Letter Contrast Sensitivity Test

Score Sheet

Patient _____ Administered by _____

Date _____ Correction _____ Test distance _____

Comments _____

Quick Instructions: Instruct patient to read letters left to right for each line, from top to bottom of the chart. Mark misses with an "X." Stop test on 2 consecutive misses.

Important: Allow *only* the letters C D H K N O R S V Z as responses.

FORM 1 Left eye ☐ Right eye ☐ Binocular ☐

C ☐ 0.04	H ☐ 0.08	V ☐ 0.12	O ☐ 0.16	S ☐ 0.20	N ☐ 0.24
D ☐ 0.28	S ☐ 0.32	Z ☐ 0.36	N ☐ 0.40	R ☐ 0.44	K ☐ 0.48
N ☐ 0.52	D ☐ 0.56	R ☐ 0.60	H ☐ 0.64	V ☐ 0.68	Z ☐ 0.72
C ☐ 0.76	S ☐ 0.80	O ☐ 0.84	N ☐ 0.88	K ☐ 0.92	H ☐ 0.96
K ☐ 1.00	N ☐ 1.04	V ☐ 1.08	D ☐ 1.12	S ☐ 1.16	R ☐ 1.20
Z ☐ 1.24	R ☐ 1.28	D ☐ 1.32	K ☐ 1.36	H ☐ 1.40	O ☐ 1.44
H ☐ 1.48	Z ☐ 1.52	C ☐ 1.56	V ☐ 1.60	R ☐ 1.64	K ☐ 1.68
S ☐ 1.72	C ☐ 1.76	Z ☐ 1.80	D ☐ 1.84	V ☐ 1.88	O ☐ 1.92

Value of final correct letter: _____

Number of misses prior to stopping _____X 0.04 = _____

Subtract

log Contrast Sensitivity _____

FORM 2 Left eye ☐ Right eye ☐ Binocular ☐

K ☐ 0.04	S ☐ 0.08	H ☐ 0.12	O ☐ 0.16	N ☐ 0.20	C ☐ 0.24
Z ☐ 0.28	D ☐ 0.32	C ☐ 0.36	R ☐ 0.40	V ☐ 0.44	O ☐ 0.48
C ☐ 0.52	K ☐ 0.56	O ☐ 0.60	N ☐ 0.64	R ☐ 0.68	S ☐ 0.72
N ☐ 0.76	S ☐ 0.80	Z ☐ 0.84	K ☐ 0.88	H ☐ 0.92	D ☐ 0.96
H ☐ 1.00	N ☐ 1.04	C ☐ 1.08	O ☐ 1.12	R ☐ 1.16	Z ☐ 1.20
V ☐ 1.24	K ☐ 1.28	S ☐ 1.32	N ☐ 1.36	D ☐ 1.40	R ☐ 1.44
K ☐ 1.48	R ☐ 1.52	V ☐ 1.56	Z ☐ 1.60	O ☐ 1.64	S ☐ 1.68
V ☐ 1.72	Z ☐ 1.76	C ☐ 1.80	D ☐ 1.84	V ☐ 1.88	H ☐ 1.92

Value of final correct letter: _____

Number of misses prior to stopping _____X 0.04 = _____

Subtract

log Contrast Sensitivity _____

FORM 3 Left eye ☐ Right eye ☐ Binocular ☐

H ☐ 0.04	R ☐ 0.08	Z ☐ 0.12	V ☐ 0.16	C ☐ 0.20	N ☐ 0.24
S ☐ 0.28	O ☐ 0.32	K ☐ 0.36	D ☐ 0.40	R ☐ 0.44	S ☐ 0.48
K ☐ 0.52	D ☐ 0.56	C ☐ 0.60	V ☐ 0.64	O ☐ 0.68	H ☐ 0.72
N ☐ 0.76	S ☐ 0.80	O ☐ 0.84	Z ☐ 0.88	C ☐ 0.92	D ☐ 0.96
R ☐ 1.00	H ☐ 1.04	N ☐ 1.08	K ☐ 1.12	Z ☐ 1.16	O ☐ 1.20
C ☐ 1.24	R ☐ 1.28	S ☐ 1.32	V ☐ 1.36	K ☐ 1.40	N ☐ 1.44
S ☐ 1.48	K ☐ 1.52	R ☐ 1.56	N ☐ 1.60	H ☐ 1.64	D ☐ 1.68
C ☐ 1.72	V ☐ 1.76	H ☐ 1.80	D ☐ 1.84	O ☐ 1.88	Z ☐ 1.92

Value of final correct letter: _____

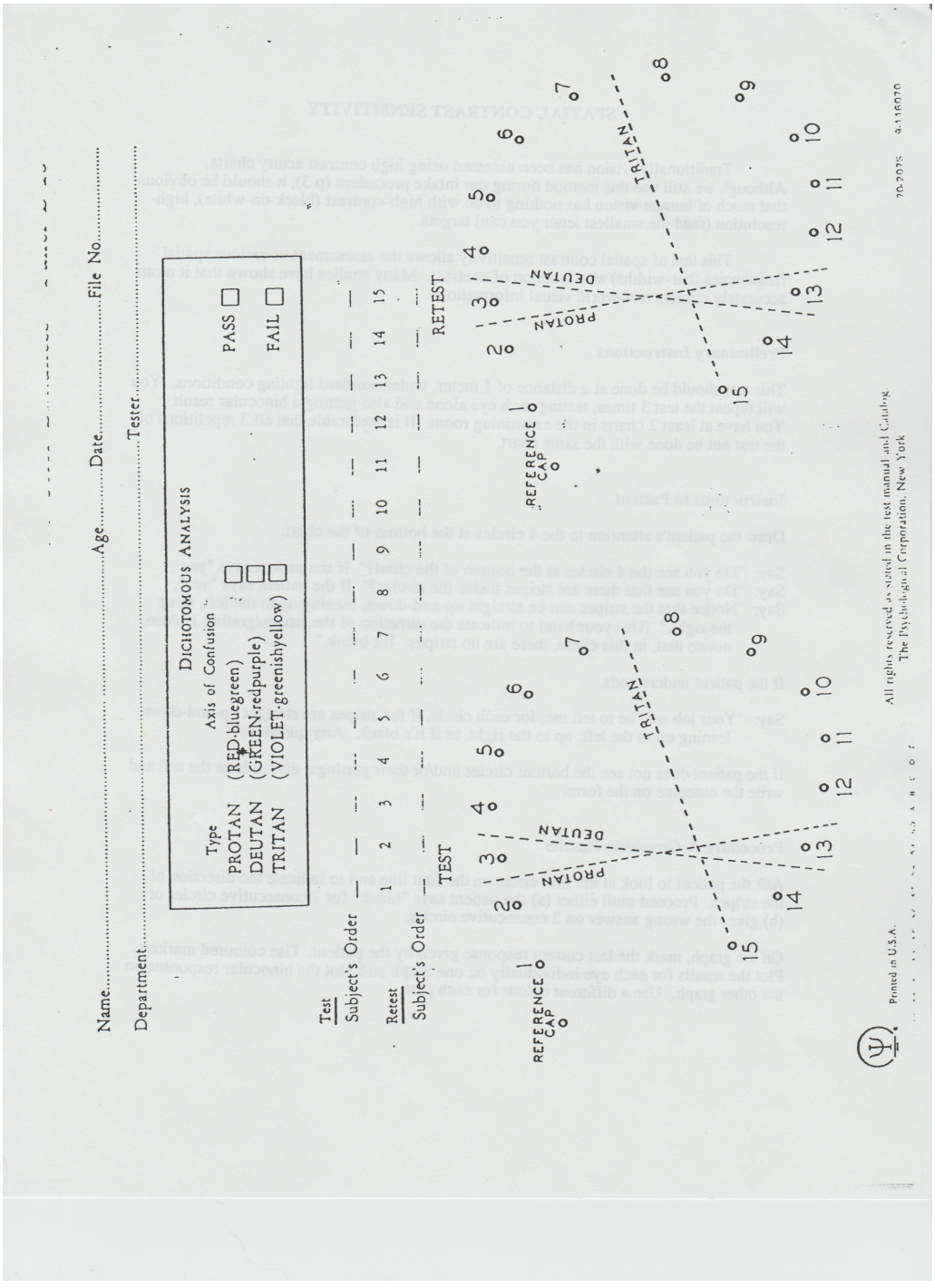
Number of misses prior to stopping _____X 0.04 = _____

Subtract

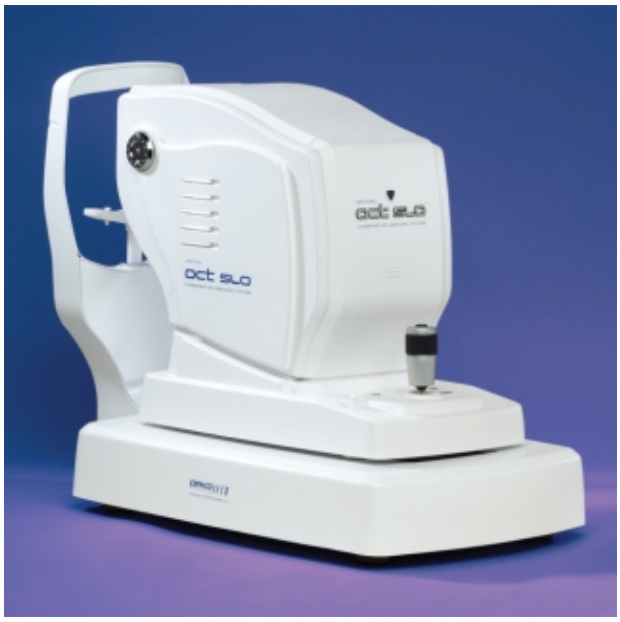
log Contrast Sensitivity _____

mars perceptrix

Farnsworth D-15 Colour Assessment

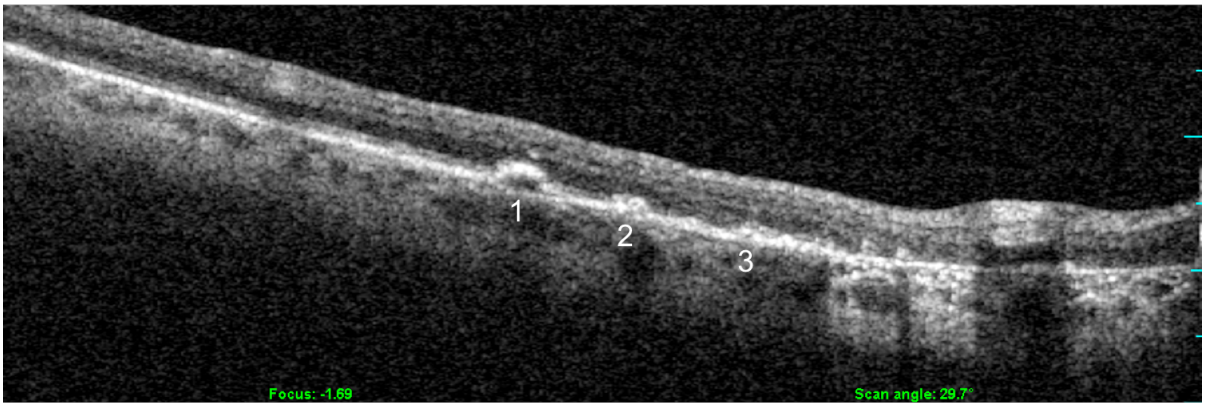


Optical Coherence Tomographer/Scanning Laser Ophthalmoscope (OCT/SLO)



The OCT function was used to take raster scans of participant eyes from which drusen characteristics were studied. The OCT function also took retinal topography scans, which allowed location of the anatomical fovea. The SLO function was used to measured fixation stability.

Example Raster Scan



1. Low reflective, convex, nonhomogeneous, foci
2. High reflective, concave, nonhomogeneous
3. High reflective, concave, nonhomogeneous

A raster scan from a retina with AMD. Three drusen are labelled according to the categories described in Khanifar et al., 2008. The scan also shows spots of geographic atrophy on the right where light passes into the choroid due to degeneration of the RPE.

Appendix C: Measures of Cognitive Function

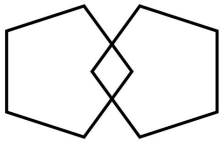
The Mini Mental-State Exam (MMSE)

MINI MENTAL STATE EXAMINATION (MMSE)

Name:

DOB:

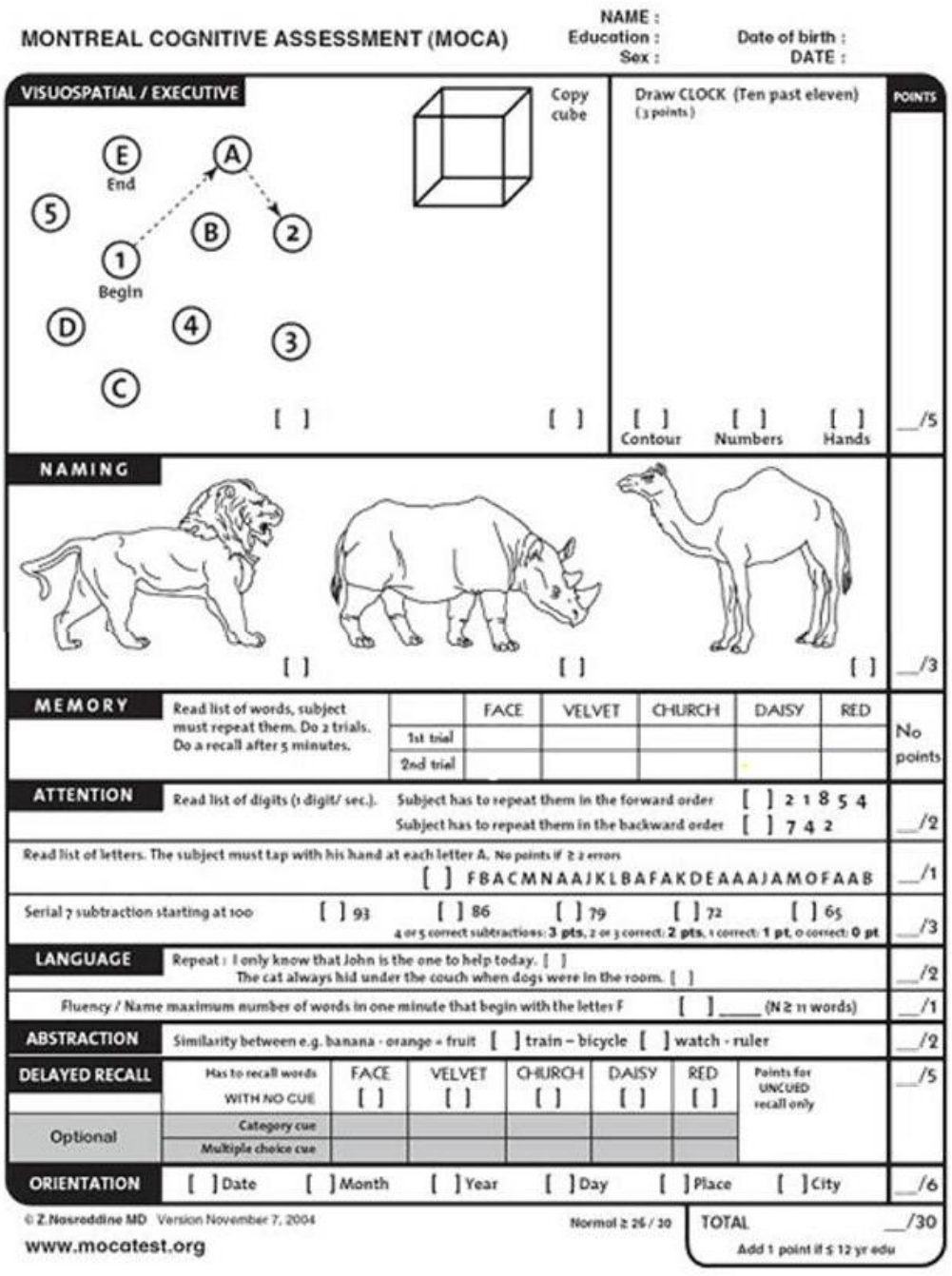
Hospital Number:

One point for each answer	**DATE:**		
ORIENTATION Year Season Month Date Time	...../ 5	...../ 5	...../ 5
Country Town District Hospital Ward/Floor	...../ 5	...../ 5	...../ 5
REGISTRATION Examiner names three objects (e.g. apple, table, penny) and asks the patient to repeat (1 point for each correct. THEN the patient learns the 3 names repeating until correct).	...../ 3	...../ 3	...../ 3
ATTENTION AND CALCULATION Subtract 7 from 100, then repeat from result. Continue five times: 100, 93, 86, 79, 65. (Alternative: spell "WORLD" backwards: DLROW).	...../ 5	...../ 5	...../ 5
RECALL Ask for the names of the three objects learned earlier.	...../ 3	...../ 3	...../ 3
LANGUAGE Name two objects (e.g. pen, watch).	...../ 2	...../ 2	...../ 2
Repeat "No ifs, ands, or buts".	...../ 1	...../ 1	...../ 1
Give a three-stage command. Score 1 for each stage. (e.g. "Place index finger of right hand on your nose and then on your left ear").	...../ 3	...../ 3	...../ 3
Ask the patient to read and obey a written command on a piece of paper. The written instruction is: "Close your eyes".	...../ 1	...../ 1	...../ 1
Ask the patient to write a sentence. Score 1 if it is sensible and has a subject and a verb.	...../ 1	...../ 1	...../ 1
COPYING: Ask the patient to copy a pair of intersecting pentagons 	...../ 1	...../ 1	...../ 1
TOTAL:	...../ 30	...../ 30	...../ 30

MMSE scoring

24-30: no cognitive impairment
18-23: mild cognitive impairment
0-17: severe cognitive impairment

The Montreal Cognitive Assessment (MoCA)



MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education :
Sex :
Date of birth :
DATE :

VISUOSPATIAL / EXECUTIVE

Copy cube

Draw CLOCK (Ten past eleven) (3 points)

POINTS

E End, A, 5, B, 2, 1 Begin, D, 4, 3, C

[ ] [ ]

[ ] Contour [ ] Numbers [ ] Hands /5

NAMING

[ ] [ ] [ ] /3

MEMORY

Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION

Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [ ] 2 1 8 5 4

Subject has to repeat them in the backward order [ ] 7 4 2 /2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B /1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** /3

LANGUAGE

Repeat : I only know that John is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ] /2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] ____ (N ≥ 11 words) /1

ABSTRACTION

Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler /2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE	VELVET	CHURCH	DAISY	RED	Points for UNCUED recall only
		[ ]	[ ]	[ ]	[ ]	[ ]	
Optional	Category cue						
	Multiple choice cue						

/5

ORIENTATION

[ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City /6

 Version November 7, 2004

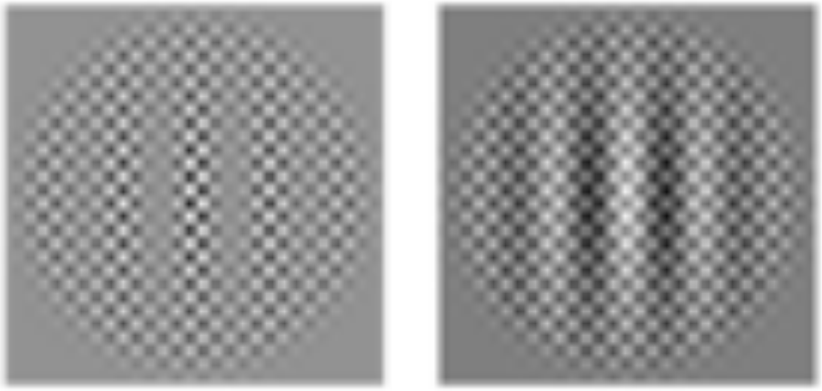
www.mocatest.org

Normal ≥ 26 / 30

TOTAL /30

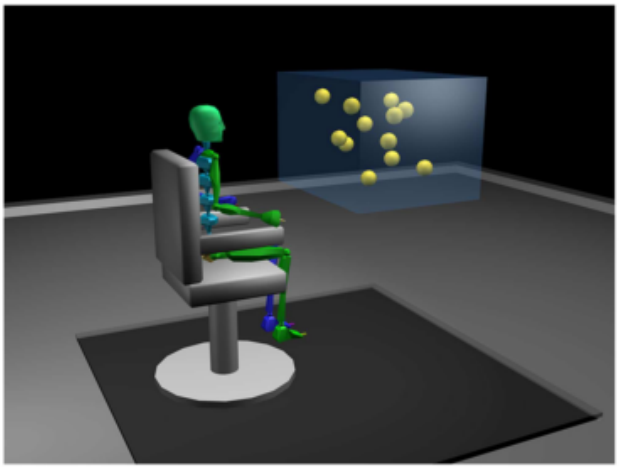
Add 1 point if ≤ 12 yr edu

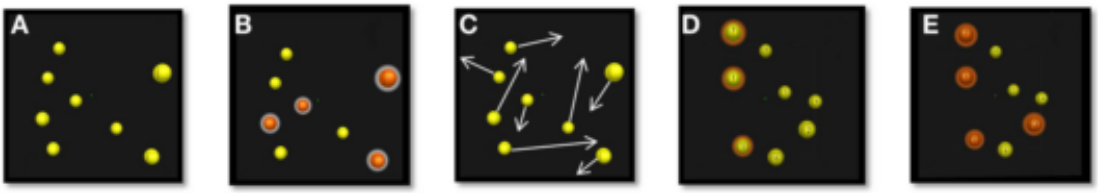
The Neurominder By Cognisens



The Neurominder by Cognisens measures mild perceptual impairment, which is thought to be the precursor to MCI. Deficits in second-order processing are suggested to be one of the initial signs of MCI. Deficits like this are too subtle to be identified by traditional questionnaires. The Neurominder uses a series of gratings to measure threshold perception of first and second-order stimuli.

Neurotracker and 3D Multiple Object Tracking (3DMOT)





The Neurotracker assesses perceptual-cognitive skills such as attention, focus and decision-making. The user is seated and asked to track the movement of targets across a 3D projection. Older adults are asked to track three objects (even though this example highlights four). Three balls are highlighted at the beginning of each trial. The highlight disappears and the targets bounce inside a cube-shaped space. Once motion stops, each ball is assigned a number. The user is asked to identify which targets were highlighted at the beginning of the trial.