The effect of complement factor H Y402H polymorphism on visual outcomes after anti-VEGF treatment of exudative AMD

Thesis

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By

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Abstract

Purpose: The Y402H complement factor H single nucleotide polymorphism (SNP) is associated with an increase in incidence of age-related macular degeneration (AMD). However, it is not well understood if this pro-inflammatory risk factor affects visual acuity outcomes after treatment with anti-vascular endothelial growth factor (VEGF). The purpose of this study was to assess the relationships among Y402H status, visual acuity (VA), and anti-VEGF treatment outcomes.

Methods: Patients with AMD receiving anti-VEGF injections were recruited from the retina service at The Ohio State University. Visual acuity was measured at the initiation of a series of anti-VEGF injections and one year later using a back-lit ETDRS chart with by-letter scoring. DNA was isolated from blood samples using Qiagen's DNeasy kit and SNP status determined with a custom TaqMan SNP genotyping assay. Regression analyses were used to assess the relationships between SNP status and outcomes.

Results: Eighty-four patients (51.2% female) were enrolled with a mean ± SD age of 81 ± 9 years. Mean VA of the treated eye was 0.75 ± 0.47 logMAR at time of enrollment. Of our patients, 22.6% did not have Y402H risk factor, 51.2% were heterozygous, and 26.2% were homozygous. Visual acuity at baseline and one year was significantly

associated with SNP status, with decreased VA for heterozygotes and a further reduction for homozygotes. Mean change in VA from baseline to one year for all subjects was 0.08 ± 0.23 logMAR. Y402H status was not associated with change in VA at one year.

Conclusions: The presence of Y402H was associated with poorer VA at baseline and one year. The lack of apparent association of Y402H status with change in VA with treatment may be due to differences in factors such as AMD stage and previous treatments at time of the baseline visit. Further work will investigate the role of these factors in addition to Y402H status in determining treatment outcomes.

Dedication

This Best Friend Thesis is dedicated to the only person as unique and special as a Best Friend Thesis is itself, Aimee Violette.

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I would like to acknowledge my advisor, Dr. Bradley Dougherty for his advice, guidance and patience throughout this project. Drs. Heather Chandler and Dean VanNasdale for serving as active members of my committee. Anthony McCoy and The Ohio State University Plant-Microbe Genomics Facility, Paolo Fadda and The Ohio State University Genomics Shared Resource, and Dr. Heather Chandler for assistance in setting up wet laboratory experiments. Finally, Dr. Frederick Davidorf, Dr. San-San Cooley, Dr. Ellen Segerstrom, and Dr. Rebecca Deffler for their role in patient recruitment.

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Publications

1. Chakuroff C, Cooley S, Segerstrom E, Davidorf F, Dougherty B. Complement Factor H Genotype and Vision in Patients Being Treated for Neovascular AMD. Paper presented to the American Academy of Optometry, November 2017, Chicago, Illinois.

2. Li J, Motlagh H, Chakuroff C, Thompson E, Hilser V. Thermodynamic Dissection of the Intrinsically Disordered N-terminal Domain of Human Glucocorticoid Receptor. Journal of Biological Chemistry, 287 (32); 2012, pp.26777-87

Fields of Study

Major Field: Vision Science

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Chapter 1 Introduction

Age-related macular degeneration as a public health concern

Age-related macular degeneration (AMD) is one of the leading causes of blindness in developed countries worldwide making the management of the disease an important public health concern. AMD is a disease of the central retina that ultimately results in central vision loss while sparing peripheral vision. Through the early stages of AMD there is usually little impact on a person's quality of life. However, as the disease progresses to the late stages, the central vision loss is so severe that there is significant functional loss and resulting depression can occur.[1]

The global prevalence of any form of AMD is reported as 8.69% of those over aged 45 years with greater occurrence in Europeans (11.2%) than Asians (6.8%) or Africans (7.1%).[2] Of those aged 40 and above, 1.63% worldwide are expected to have vision threatening choroidal neovascularization (CNV), geographic atrophy (GA), or both.[3] In the United States, the 2000 census estimated 1.75 million people over the age of 40 years to have AMD with another 7.3 million with large drusen in at least one eye, putting them at high risk of developing AMD.[4] Disease becomes more prevalent with increasing age with a meta-analysis from three pooled studies indicating by the age of 85, 13.05% of participants had at least one lesion.[3] With the rapidly aging American population, cases of disease are expected to more than double from 1.75 to 2.95 million

by 2020 making AMD an increasingly important public health concern.[4] The disease also seems to affect fair-skinned, female individuals preferentially with 15% of American women over the age of 80 having vision threatening geographic atrophy, neovascular AMD, or a combination of the two.[4] Other studies have indicated there is no gender relationship to disease.[2,3]

Clinical signs of AMD

Typical early clinical signs of AMD include changes in the outer retina, retinal pigment epithelium (RPE), Bruch's membrane, and choriocapillaris. A common feature of AMD is drusen which can be classified as one of three main types – hard, soft, and basal laminar. These drusen are thought to develop from the RPE cells as membranous RPE material buds off from the RPE into the sub-RPE space. Hard drusen are small, dull yellow deposits that usually contain a large amount of hyaline. These can be commonly found in younger patients and are not indicative of AMD.[5] Soft drusen are pale yellow with less defined edges and are associated with an overlying detachment of the RPE basement membrane from the rest of Bruch's membrane. Histologically, initial stages of these drusen can be found as basal linear deposits within the inner collagenous layer of Bruch's membrane.[6]These soft drusen are most associated with AMD as they create a potential space for CNV and can lead to RPE atrophy as seen in GA, especially if they calcify and dehydrate.[5,7] Finally, basal laminar drusen are small, white deposits of proteins that form over regions of thickened Bruch's membrane, however, they do not predispose to AMD.[5] Macular mottling, or pigmentary changes in the macular region, is

commonly observed in AMD due to the accumulation of lipofuscin in RPE cells causing depigmentation, pleomorphism, and ultimately atrophy of the RPE cells.[7] Drusen or pigmentary changes themselves do not directly result in vision loss; however, they can promote subsequent RPE and photoreceptor atrophy. When visual acuity (VA) drops to 20/30, in addition to the presence of soft drusen, pigmentary changes, or both, the patient is typically diagnosed with early non-exudative AMD.[5] However, there are a variety of definitions for when to diagnose early AMD as well as how to stage the disease.[8]

Significant vision loss occurs with progression to one of two late disease stages. These two forms of AMD are not mutually exclusive and may occur on their own or in combination over the course of the disease. The first is choroidal neovascular AMD (also known as exudative AMD or wet AMD), which occurs as new choroidal blood vessels grow and subsequently break, leaking blood and debris into the neural retina. This results in scarring of the retina, ultimately forming a vascularized disciform scar and loss of vision. The other late stage of AMD is GA which does not involve the development of new blood vessels but is characterized by severe degeneration and atrophy of the RPE, choriocapillaris, and photoreceptors also resulting in vision loss.[9] Exudative AMD makes up about 10% of AMD cases, but is responsible for 90% of legal blindness caused by AMD.[10] In fact, the major cause of blindness in all American Caucasians over the age of 40 is AMD, accounting for 54.4% of cases, with lower occurrences of disease induced blindness in black or Hispanic populations.[11] For these reasons, understanding how to best prevent wet AMD and control its progression with treatment would have a major impact on AMD patients' quality of life.

Potential models of pathogenesis

There have been an enormous number of molecules thought to be involved in the RPE and photoreceptor degeneration characteristic of AMD. Consequently, it has been difficult for a consensus to be reached on a single mechanism that is responsible for the pathogenesis of the disease. One of the most well accepted mechanisms of AMD development involves the dysfunction of RPE cells. The RPE cells normally play a metabolic role in the regeneration of the vitamin A based chromophore that absorbs light in photoreceptors as well as the degradation of photoreceptor disks shed from the outer segment. Particularly in the macula, where photoreceptors are densely packed, the aged RPE cells lose lysosomal function and develop buildup of lipid-based debris from the membranous photoreceptor outer segment disks.

One mechanism by which RPE damage is thought to occur is through oxidative stress and reactive oxygen species (ROS) generation. Lipofuscin is thought to be a product of the auto-oxidative process and, as it sits in the RPE or Bruch's membrane, its exposure to light and high oxygen concentrations results in the release of more ROS.[12] Further damage to the RPE cells and the overlying photoreceptors occurs due to this ROS generation, ultimately causing progression of AMD. Additionally, oxidative stress stimulates RPE cells to release vascular endothelial growth factor (VEGF)-A which stimulates the growth of CNV.[13] This role of oxidative stress is the basis behind the development of an antioxidant rich nutritional supplement to slow the progression of AMD, as tested in the Age-Related Eye Disease Study (AREDS).[14] Other proposed

mechanisms for AMD include altered autophagy within the RPE which may be due to or may cause lipofuscin, accumulation of amyloid-beta plaques similar to neurodegenerative diseases, mitochondrial DNA damage in the RPE, and choroidal vascular changes similar to those seen in atherosclerosis.[15–18]

With the recent increase in genetic analysis of AMD patients, the role of inflammation in AMD pathogenesis has become more apparent as variations in complement proteins and chemokine receptors being commonly reported genetic risk factors for AMD. Further evidence of an inflammatory role in AMD pathogenesis comes from the identification of complement proteins, inflammatory acute phase reactants, and degraded inflammatory cells within drusen (See Figure 1.1).[19–21] How AMD inflammatory models relate to the oxidative stress models previously discussed has yet to be elucidated, although it is apparent that AMD pathogenesis is likely a complicated and multifaceted process.

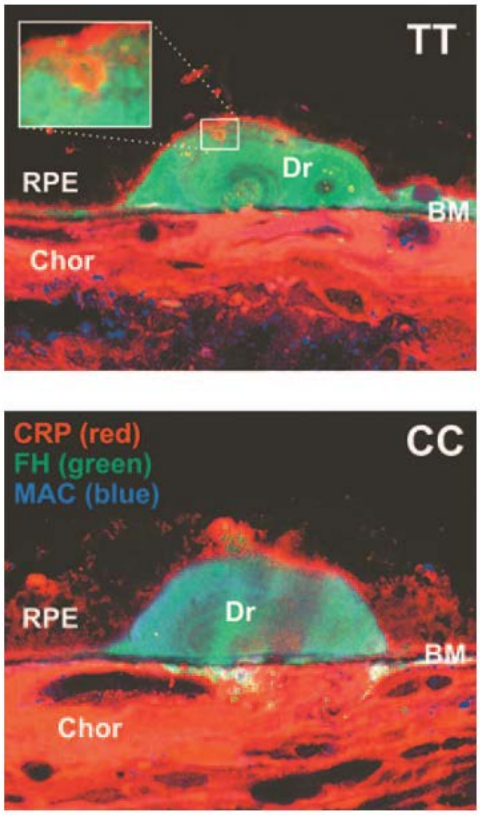

Figure 1.1 Contents of drusen in AMD. Confocal microscopy of C-reactive protein (CRP) in red and complement factor H (CFH) in green in AMD patients with TT (no risk) or CC (homozygous rick allele) Y402H CFH genotypes. Figure from Laine et al.[22]

Inflammatory models of pathogenesis

An introduction to the inflammatory system

The inflammatory process serves an important function in detecting damaged structures or foreign substances in the body that could be detrimental to the health of a tissue. Through inflammatory cascades, nearby cells are alerted to the potential threat and

the body works to remove the causative factor, ultimately facilitating the tissue regeneration process. This entire process is tightly regulated because uncontrolled inflammation can inadvertently lead to damage to healthy tissue as observed in autoimmune diseases. The two main branches of the immune system are the innate and the antigen-specific immune systems, and both, when activated, lead to an inflammatory response. The innate immune system is nonspecific and responds to broad groups of microbes, toxins, or cellular debris from trauma. The cells most commonly activated in the innate immune system include macrophages and neutrophils. The antigen-specific immune system is acquired over a lifetime as the body learns non-self proteins called antigens through the activation of T and B cell lymphocytes.[9]

The inflammatory process must be amplified by a series of mediators to be clinically observed as inflammation. These mediators include a variety of molecules such as angiogenic factors, complement factors, kinins, fibrin, histamine, prostaglandins, eicosanoids, and leukotrienes. All these mediator's act on recruitment and activation of other cells within the immune system, some of which may play an important role in AMD pathogenesis. Important immune cells within the retina include macrophages and microglia, resident macrophages in retinal tissue, which primarily phagocytose debris and pathogens and act as antigen presenting cells to activate T lymphocytes. A closely related cell to the macrophage is the dendritic cell; these cells primarily act as antigen presenting cells to activate T lymphocytes. Finally, mast cells are granular leukocytes that are generally activated through an IgE mediated pathway. When they degranulate they are

able to cause tissue damage as well as promote angiogenesis, similar to that found in neovascular AMD.[9]

Many of these inflammatory elements are found to be elevated in patients with AMD, explaining the multiple potential models associated with an inflammatory disease mechanism.

Complement mediated model

The complement cascade is a collection of over 30 different proteins made by the liver, and locally by RPE cells, to help to mediate the immune system.[21,23] The cascade can be activated through three main pathways: the classical, alternative, and lectin-mannose pathways (See Figure 1.2). Each pathway results in the activation of complement factor 3 (C3) via C3 convertase to active C3a and C3b. As the cascade perpetuates and activates other complement factors, the results include tagging pathogens for phagocytosis, producing the membrane attack complex (MAC) that lyses infected cell membranes, and creating inflammatory mediators called anaphylatoxins that attract immune cells and increase vasculature permeability.[21] As debris accumulates between the RPE cell and its basement membrane and within the inner collagenous zone of Bruch's membrane, these intracellular substances in an extracellular environment promote an inflammatory response as "atypical" activators of the alternative complement pathway. Acute phase reactants like C reactive protein (CRP) and serum amyloid P can also bind to this debris and amplify complement activation. Continued inflammatory activation and deposition of inflammatory proteins and cellular debris results in the expansion of drusen.

These drusen physically limit waste and nutrient transport between RPE cells and choriocapillaris through Bruch's membrane causing further damage to nearby RPE cells. Additionally, as the complement system is activated, the surrounding RPE cells are common targets of MAC degradation causing RPE cell functional loss and death.[24] Specifically, complement components C3a and C5a have been shown to increase RPE-induced VEGF expression providing a plausible model for how drusen contribute to the development of CNV.[20]

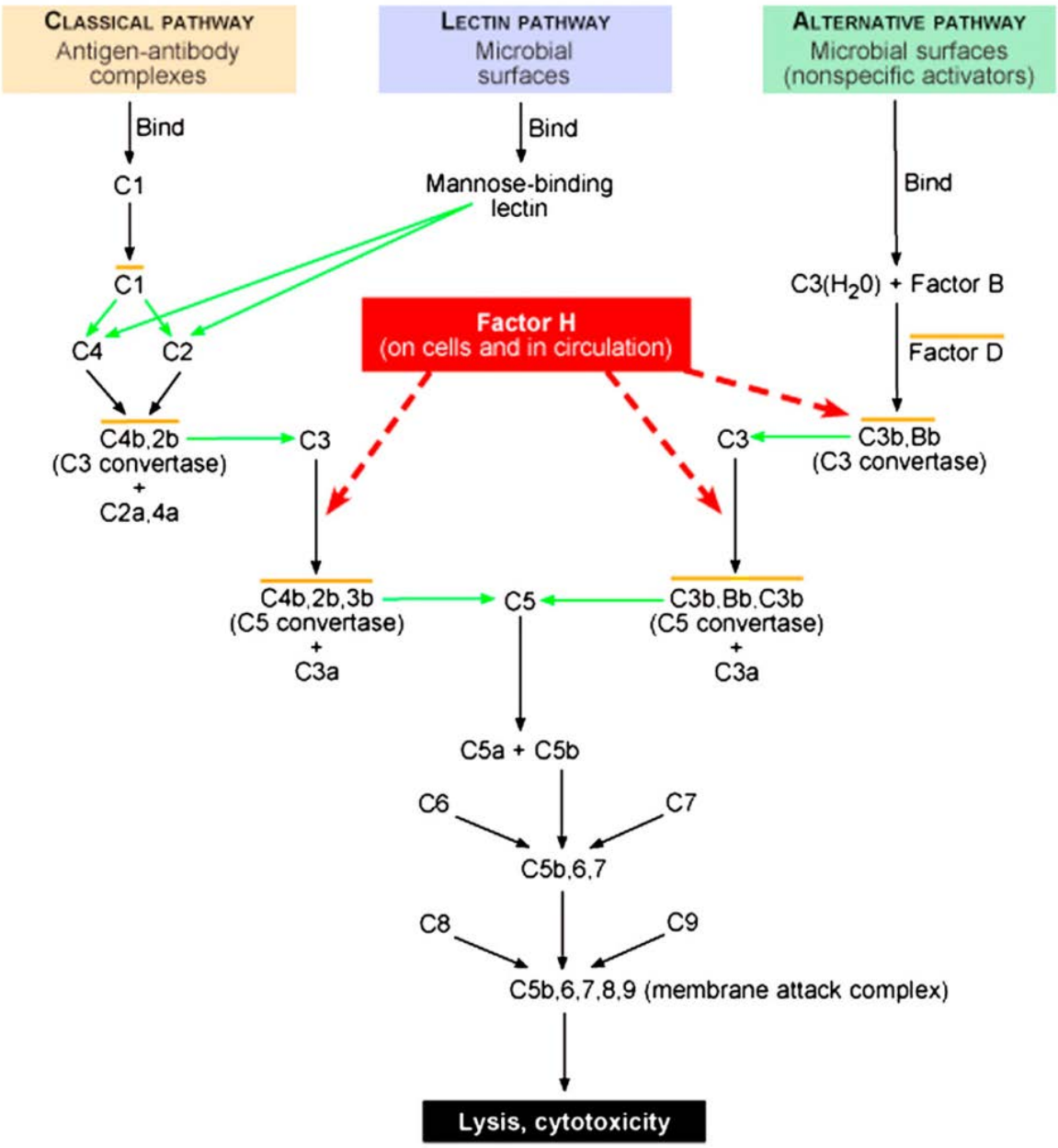

Figure 1.2 The three complement factor pathways. There are three routes of activating the complement cascade: classical, lectin, and alternative pathways. Complement factor H, in red, works to inhibit these pathways in a regulatory fashion. Figure from Donoso, et al.[25]

Other inflammatory models

Beyond the complement system, other inflammatory processes have been cited in the pathogenesis of AMD, validating the inflammatory nature of the disease. One proposed mechanism involves inflammasomes, which are collections of proteins part of the innate immune system and, when activated, allow for the maturation of interleukins. In AMD, NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasomes in RPE cells activate interleukins IL-1b and IL-18.[26,27] These interleukins induce cell death, GA, and CNV.[28,29] Potential inflammasome activators include drusen, blue light, and iron toxicity, making these factors interesting future targets for AMD pathogenesis study.[30–32]

The immunovascular model of pathogenesis takes into account age related choriocapillaris atrophy that alters shear stress on these vessels. In AMD, this atrophy seems to occur in regions that later develop RPE cell loss.[33] Endothelial cells respond by activating NLRP3 inflammasomes and the complement cascade, as previously discussed.[34,35] Gelfand et al. proposed that an increase or decrease in shear stress that is induced locally by choriocapillaris vascular changes predicts whether CNV or GA develops in AMD. In regions of decreased localized shear stress, the endothelial cells initiate a greater immune response that ultimately leads to CNV formation. In nearby regions of increased shear stress, the immune response is diminished preventing vasculature remodeling causing GA.[18]

There is also some indication that the adaptive immune response is involved in AMD pathogenesis, as noted through the production of autoantibodies and elevation of T

cells in AMD patients. Levels of anti-retinal autoantibodies are higher in patients with AMD, as measured by titers of blood serum.[36] These autoantibodies are created against all layers of the retina, but specifically have been identified against RPE proteins and drusen components such as elastin, heparan sulfate, and collagen that have been oxidized. These oxidized forms are not recognized by the immune system as self, and subsequently generate an immune response such as complement activation and immune cell recruitment.[37] It is not clear if these antibodies are a result of the AMD pathogenesis in response to stress on the tissue or if they are a mechanism causing disease. Either way, once autoantibodies are present, they likely contribute to the progression of the disease state.[38,39]

Finally, low level, chronic, and dysregulated inflammatory responses described by parainflammation and immunosenescence could be an initiating factor in AMD pathogenesis. Parainflammation is defined as a subclinical state of inflammation that is acting as an adaptive response to a noxious stimulus. In a normally aging retina, parainflammation exists as a response to oxidative stress as debris accumulates in the RPE cells and Bruch's membrane. This results in the activation of complement, production of autoantibodies, and recruitment of macrophages that promote damage to the RPE cells and potentially CNV.[40] Parainflammation also includes the activation of microglia that then migrate into the subretinal space to induce RPE apoptosis.[41] Immunosenescence describes the changes in the immune system that normally occur with age. These changes do not necessarily describe a loss of function, but a slow and altered immune function when compared to younger individuals. Immunosenescence includes a

shift in the T cell pool from naïve to mostly memory T cells and a decreased responsiveness of B cells.[27] Further, during immunosenescence there is a general increase in autoantibodies, which may bind retinal proteins and initiate an immune response leading to AMD.[42]

Pathogenesis conclusion

The exact pathogenesis of AMD has not been fully revealed; however, it appears that the inflammatory response likely plays a role through one or multiple mechanisms. Additional studies into the role of the immune system in AMD will facilitate the development of new treatments for the disease that can target these pathways. Phase III clinical trials are currently underway for Lampalizumab, which targets Factor D in the complement cascade to treat GA.[43] POT-4 is an inhibitor of C3 convertase activation that was used in a phase I clinical trial, although 93% of patients had no improvement in VA.[44] Broad anti-inflammatory agents including triamcinolone acetonide and fluocinolone acetonide corticosteroid implants have also been used in clinical trials, but neither has been shown to improve VA.[44] Some progress has been made in developing new AMD treatments and hopefully new treatments for GA and neovascular AMD treatment will be available soon.

AMD risk factors

There are likely multiple pathways involved in AMD pathogenesis and as such, it is logical that a wide variety of risk factors have been implicated in the disease. The most

apparent risk factor in this age-related degeneration is not surprisingly age, with a meta-analysis of participants in the Beaver Dam Eye Study, Rotterdam Eye Study, and Blue Mountain Eye Study demonstrating that the prevalence of AMD in those 55-65 years age was 0.2%; the prevalence of AMD jumped to 13% in those older than 85 years.[3] AMD has also been found more commonly in Caucasian populations and those with a family history of the disease.[45–47] The most consistently identified modifiable risk factor for AMD is smoking with an average of 2 to 3.5 times increased risk of disease incidence for current smokers.[3,46] Other risk factors that have been shown to affect AMD, though not as consistently, include a body mass index over 25, greater sunlight exposure, and cardiovascular disease including hypertension, hypercholesterolemia, and diabetes.[46,48–50] A meta-analysis of 18 studies showed no significant difference in late AMD incidence between men and women, although the Blue Mountain Eye Study demonstrated that women had a statistically significant increased risk of AMD, especially women who had lower estrogen due to a shorter duration of menarche.[46,51] A diet high in fat, either animal or plant based, has been shown to increase the progression of AMD; however, eating fish and foods rich with omega-3 fatty acids has been shown to decrease the risk of progression.[52,53] As new AMD treatments are developed, it is helpful to determine which factors place an individual at highest risk of disease onset and progression so that clinicians can identify which patients need earlier treatment.

Genetic risk factors

Since having a family history of AMD has been shown to put a patient at nearly a four times increased risk of the late stages of the disease, it seems likely that genetic risk factors could have a role in determining who develops AMD and its subsequent course.[47] The biggest breakthrough in understanding AMD genetics came from Klein et al. in 2005 when the group completed a whole-genome association study which compared over 105,000 single nucleotide polymorphisms (SNPs) between patients with AMD and controls.[54] A SNP is a single base change in the nucleic acid code that occurs naturally in >1% of the general population. The identification of SNPs and their analysis has become the primary method of genetic analysis in modern genetic studies. Although some SNPs result in no change in amino acid sequence and ultimately protein function, SNPs may still alter the regulation of gene transcription and ultimately function. Studies of SNPs in populations of disease can help determine which SNPS might be significant for disease occurrence.[55] The Klein et al. study identified a SNP in complement factor H (CFH) position reference SNP (rs)1061170 that increased the risk of AMD by a factor of 4.6 if the individual was heterozygous and 7.4 if homozygous.[54] Since this study, numerous SNPs have been identified, either for promoting or protecting from AMD development. A table adapted from Black et al. includes a list of the most consistently linked genes to AMD, including those identified by the AMD Gene Consortium's 2013 analysis of over 2.4 million SNPs (See Table 1.1).[56,57] The latest whole-genome analysis was completed in 2015 by the International AMD Genomics Consortium. They analyzed greater than 12 million SNPs in over 40,000 subjects resulting in a list of 52 genomic variants, some very

rare, that they estimate account for about 50% of all AMD heritability.[58] Interestingly, rs1061170 did not make the short list for either of these analyses, indicating it was not as specific to AMD as other SNPs.

Gene	Pathway/Function Implicated
ADAMTS9/MIR548A2	Proteoglycan cleavage, inhibition of angiogenesis
APOE	Lipoprotein metabolism, atherosclerosis
ARMS2/HTRA1	Unknown, possibly mitochondrial/cell growth
B3GALTL	Glucose transport
C2/CFB	Complement
C3	Complement
CETP	Lipoprotein metabolism, atherosclerosis
CFH	Complement
CFI	Complement
COL10A1	Atherosclerosis
COL8A/FILIP1L	Extracellular matrix/angiogenic activity of endothelial cells
IER3/DDR1	Cell death/growth
LIPC	Lipoprotein metabolism, atherosclerosis
RAD51B	Homologous recombination
SLC16A8	Lactate transport
TGFBR1	Widespread, including angiogenesis
THFRSF10A	Cell death
TIMP3	Extracellular matrix degeneration
VEGFA	Angiogenesis

Table 1.1 Genes affected in AMD adapted from Black et al. [56]

With all this new genetic information, the question arises if there is any translational benefit to patient care. Although a few commercial genetic tests for AMD exist, the American Academy of Ophthalmology does not currently recommend genetic testing as the standard of care. This is partly due to inaccuracies of the commercially available tests, but also because the determination of someone's genetic risk, at this time, does not result in changes in patient management that ultimately alters disease outcome.[57] Although genetic risk factors can help clinicians determine who is at higher risk of disease, until we develop better interventions for AMD or understand how genetic factors alter our current treatments, knowing someone's genetic risk is arguably not clinically relevant.

Complement factor H

The rs1061170 loci of CFH remains the most well-studied of the AMD risk factors. Since its discovery in 2005, PubMed shows 340 publications regarding the relationship between CFH and AMD and research is ongoing as this protein plays an important part in regulating the complement system (PubMed search: (((rs1061170) OR (Y402H)) AND ((macular degeneration) OR (AMD)))).

The complement system must remain under tight control because too much activation can result in damage of healthy tissues as inflammation runs unregulated, while too little activation results in tissues susceptible to infection.[59] For this reason, it is not surprising that over 20 complement regulatory proteins, including CFH, have been identified.[59] CFH is the best characterized protein of the CFH family of seven regulatory

proteins. All of these proteins are produced in the liver and are coded by the CFH gene cluster on chromosome 1q32.[60] Complement factor H consists of 20 short consensus repeats (SCRs) about 60 amino acids each that form globular structures (See Figure 1.3).[61] The N-terminal domain consisting of SCRs 1-4 is responsible for the complement regulatory function of CFH by acting as a cofactor for C3 convertase disassembly to inhibit the alternative pathway (See Figure 1.2).[62,63] Short consensus repeats 1-5, 12-14, and 19-20 contain binding sites to three distinct binding sites on complement factor C3b and its cleavage fragments.[64] Through these interactions, CFH works to competitively bind C3b to prevent its involvement in the alternative pathway complement cascade and slow the inflammatory process.[64] Since the classical pathway of complement activation includes amplification through the alternative pathway, CFH mediation of complement activation affects both pathways.[65] Complement factor H has also been found to bind to heparin, CRP, neutrophils, and microbial ligands on pathogens indicating that it might serve multiple functions in the immune response.[60,66] CFH is highly associated with AMD pathogenesis because although it contains the relatively common rs1061170 SNP, CFH has also been shown to have a high burden of other rare AMD risk SNPs.[58] However, there are also variations in CFH that result in protection from AMD such as the deletion of CFHR1 and CFHR3.[60] Outside of AMD, dysfunction of CFH has been implicated in atypical hemolytic uremic syndrome and membranoproliferative glomerulonephritis type II. Only a few CFH SNPs are associated with AMD and atypical hemolytic uremic syndrome, but more similarities, including rs1061170, exist between AMD and membranoproliferative glomerulonephritis type II. In fact, patients with

membranoproliferative glomerulonephritis type II have been shown to develop drusen-like changes in the macular region.[67]

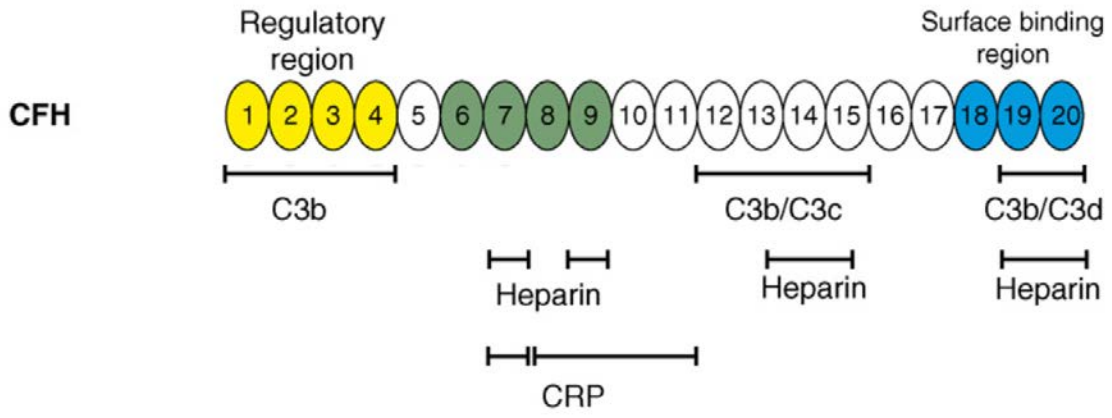

Figure 1.3 The 20 domains of CFH. Figure adapted from Józsi and Zipfel[60]

The rs1061170 or the Y402H polymorphism of CFH

In individuals with the rs1061170 SNP, a single base nucleic acid change of a T to a C nucleotide results in an alteration of a tyrosine to a histidine in position 402 of the CFH protein (See Figure 1.4).[54] The 402nd (384 of the mature polypeptide) amino acid change occurs in SCR 7, within a region of five positively charged amino acids (positions R369, K370, R386, K387, K392). This region has been shown to be involved in CFH's

ability to bind heparin, CRP, and M protein of bacterial antigens.[68] It was hypothesized that the substitution of a positively charged histidine for a neutral tyrosine in the region of SCR 7 would repel these positively charged amino acids necessary within the binding site.[69] Y402H has been shown to reduce the ability of CFH to bind to CRP and M protein, although only the truncated CFHL-1, another member of the CFH family, was affected in heparin binding.[22,70] However, another study demonstrated that Y402H resulted in a reduced ability for full length CFH to bind CRP, heparin, and RPE cells.[71] Additionally, Y402H has been shown to decrease the ability of CFH to bind to heparan sulfate in Bruch's membrane.[72] If Y402H impairs CFH to bind to RPE cells and Bruch's membrane, its inability to locally halt the alternative pathway of complement activation in these regions would cause the local inflammation that results in damage and drusen within these structures.

Interestingly, Y402H does not seem to affect the binding of CFH to C3b, the primary ligand of CFH.[71] Therefore, it seems the main pathogenesis in Y402H SNP is due to impaired binding with other molecules that regulate CFH activity. The interaction between CFH and CRP is of particular interest as we continue to decipher the exact role of Y402H in AMD pathogenesis. Both elevated CRP (>3mg/L) and Y402H have been shown to independently increase the risk of AMD, and when both are present, the risk of advanced AMD, GA, and CNV increases further.[73] Another study demonstrated that elevated CRP and Y402H have an additive effect on advanced AMD risk, suggesting that perhaps the reduced binding affinity between Y402H CFH and CRP results in even poorer complement regulation that either factor on their own.[74] There is conflicting

evidence on whether CRP increases or has no effect on the ability of CFH to bind and inhibit C3b.[75,76] If the former is true, then the loss of CRP interactions in Y402H would result in reduced ability of CFH to inhibit C3b. In the latter, it is postulated that reduced CRP binding, like that which occurs with Y402H, still results in reduced complement regulation since CRP less effectively binds and localizes CRP to regions where an inflammatory response is desired.[75,76]

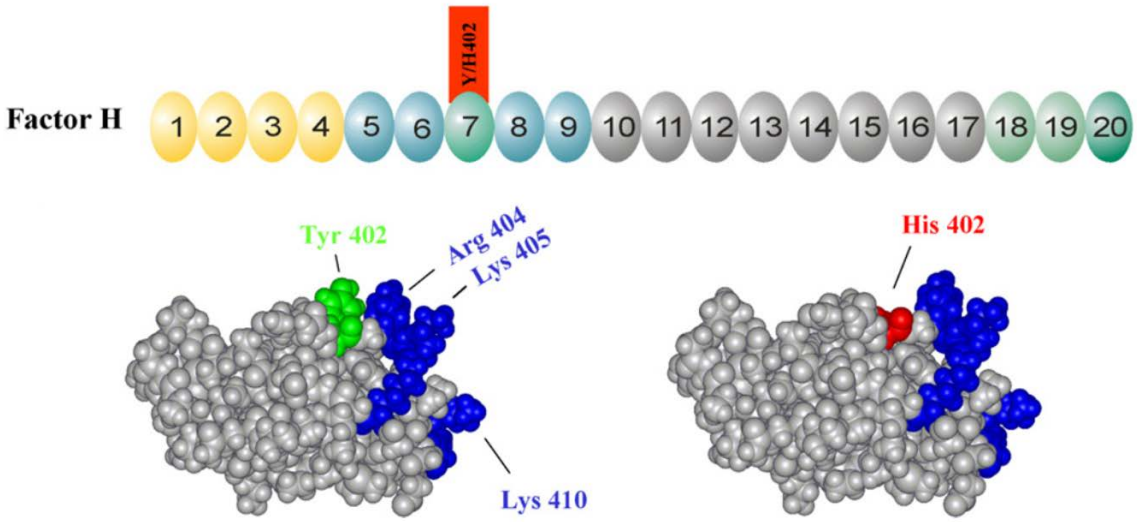

Figure 1.4 Location of Y402H SNP in linear and 3D structure of CFH. The 3D structure on the left is that of the wild type CFH. On the right is the Y402H conformation. Figure adapted from Skerka et al.[71]

Although the exact mechanism of how the Y402H SNP results in increased AMD risk is unknown, many studies have shown this association exists and that the risk is dose dependent; risk increases depending on if an individual has one or two higher risk C alleles. In studies of AMD prevalence, there is a 1.5 to 2.5 times increased risk of disease for those with one C allele, and anywhere from a 2.1 to 6.3 times increased risk for those with two copies of the C allele.[77–80] In those who already have the disease, the risk of progression is greater for those with two C alleles and the odds of having late stage disease is 11 times greater in those with the CC genotype than those with TT.[48,81,82] There is also evidence that the elevated risk due to Y402H is further elevated by smoking, high BMI, and elevated erythrocyte sedimentation rate (ESR) levels.[78,82] There are conflicting results regarding whether the presence of Y402H results in an earlier age of AMD onset.[78,83]

What makes the study of Y402H especially important is that it appears frequently in the general population. Although other studies have since identified numerous SNPs that have a greater risk for AMD occurrence, the relative rarity of these SNPs in the general population make them less useful as potential therapeutic targets.[56] The Rotterdam Eye Study found the high risk Y402H C allele frequency to be 36.2% in their population with a population attributable disease risk of 54.0%. [82] These values are reflected in most studies of the Caucasian population with an average gene frequency of approximately 30% in the general population and 60% in those with AMD, and a

population attributable risk of 50%; however, the frequency is lower in non-Caucasian populations.[25,78,79,84]

AMD treatment

The natural history of AMD typically results in continued loss of central vision, especially in the advanced forms. From the Age-Related Eye Disease Study (AREDS), it was found the rate of progression to an advanced form of AMD increased with the initial severity of the disease, increasing age, smoking, and being female. Of those at the highest risk of advancement in the study, most severe AMD at baseline and oldest age (75-80 years), 26% developed GA and 48% CNV over 10 years. In eyes that never developed the advanced stages of disease, at 10 years the average visual acuity was 20/25; in those who progressed to CNV or GA, the average VA was 20/200 in the affected eyes.[85] Unfortunately, current treatments are somewhat limited for AMD. For those in the early stages of the disease the primary treatment is to reduce risk factors such as smoking, and potentially reducing sun exposure and losing weight. The AREDS recommended combination of antioxidants and macular pigments in the AREDS 2 formula were shown to have a 25% reduction in the progression of intermediate and advanced AMD.[14,86] There are currently no additional treatments for GA beyond AREDS 2 supplementation, however, there are a few options for CNV. A summary of the major studies that have evaluated CNV treatment is included in Table 1.2.

Study Name	Duration of follow up (years)	Treatment	Control	Major Outcome	Citation
VISION	3	Pegaptanib 0.3 or 1.3 mg every 6 weeks	Placebo	Pegaptanib was well tolerated with rare serious adverse events and better acuity outcomes than sham injections	Gragoudas et al. 2004[87]; Singerman et al. 2008[88]
ANCHOR	2	Ranibizumab 0.5 mg monthly	Photodynamic therapy (PDT)	Ranibizumab had statistically significant and clinically meaningful improvement in VA outcomes and fluorescein angiography lesion changes over PDT	Brown et al. 2009[89]
MARINA	2	Ranibizumab 0.3 or 0.5 mg monthly	Placebo	Ranibizumab preserved vision over 2 years (net gain of letters) with rare serious adverse events	Rosenfeld et al. 2006[90]
HARBOR	1	Ranibizumab 0.5 or 2.0 mg monthly or PRN	N/A	All groups achieved clinically significant gains in VA with PRN dosing requiring 4 fewer injections	Busbee et al. 2013[91]
SUSTAIN	1	Ranibizumab 0.5 mg monthly for 3 months then PRN	N/A	Best gain in VA occurred at 3 months, declined slightly, but then held stable with PRN dosage	Holz et al. 2010[92]
PrONTO	2	Ranibizumab 0.3 mg monthly for 3 months then PRN	N/A	Using an OCT to guide PRN treatment resulted in equivalent VA outcomes to monthly injections with fewer injections required	Lalwani et al. 2009[93]
SAILOR	1	Ranibizumab 0.5 mg monthly for 3 months then PRN based on OCT or physician's discretion	N/A	Both dosing regimens had a net gain in VA	Boyer et al. 2009[89]

Table 1.2 Summary of major studies investigating neovascular AMD treatment

SANA	0.25	Bevacizumab systemically	N/A	Average gain of 14 letters in VA and treatment well tolerated ocularly, however intravitreal administration would be safer long term	Moshfeghi et al. 2006[94]
IVAN	1	Bevacizumab 1.25 mg monthly or PRN	Ranibizumab 0.5 mg monthly or PRN	Both drugs exhibited equivalent safety and efficacy, though VA results were inconclusive. Bevacizumab was less costly.	Chakravarthy et al. 2012[95]
MANTA	1	Bevacizumab 1.25 mg monthly for 3 months then PRN	Ranibizumab 0.5 mg monthly for 3 months then PRN	Both drugs had equivalent gain in VA letters	Krebs et al. 2013[96]
CATT	2	Bevacizumab 1.25 mg monthly or PRN	Ranibizumab 0.5 mg monthly or PRN	Both drugs had equivalent effects on VA, less gain in VA occurred with PRN dosage, bevacizumab potentially has higher rates of serious adverse events but inconclusive	Martin et al. 2012[97]
VIEW 1 and 2	2	Aflibercept 2 mg monthly for 3 months then PRN	Ranibizumab 0.5 mg monthly	Aflibercept every 2 months was non-inferior to ranibizumab monthly	Heier et al. 2012[98]

Table 1.2. Summary of major studies investigating neovascular AMD treatment (continued)

Historically, the original method for treating CNV lesions was argon laser photocoagulation which was first used in the early 1980s. This was shown to be marginally better than no treatment for extrafoveal lesion (>200 μm from foveal avascular zone), but unfortunately, 47% of treated patients lost 6 or more lines of VA 3 years after treatment.[99] Additionally, the treatment itself resulted in a destruction of the tissue leaving a scotoma. Photodynamic therapy came about in the late 1990s. A

compound called verteporfin is injected intravenously and allowed to pool in neovascular membranes of CNV. The retina is then exposed to infrared light which activates verteporfin to form free radicals in those vessels causing them to atrophy.[21] This treatment was better at stabilizing vision compared to laser photocoagulation; however, the average visual change over two years was still a loss of 13 letters with the therapy compared to 19 letters without treatment in those with classic CNV.[100]

The latest advancement, and the new standard of care, for CNV treatment is anti-VEGF drugs which target VEGF promotion of choroidal vasculature growth in CNV. Macugen was approved by the FDA in 2004 and in initial trials, 33% of patients maintained or even regained some VA as compared to 22% receiving sham injections.[101] However, Macugen is no longer in use with the advent of three new anti-VEGF medications with better visual outcomes. The first product to come onto the market was Lucentis (ranibizumab) which, in the Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular Age-Related Macular Degeneration (MARINA) trial, showed an average gain of 6.5 or 7.2 letters, depending on the dose, compared to a loss of 10.4 letters in sham injections. Furthermore, the benefit to VA was stable for 24 months of follow up.[90] The American Academy of Ophthalmology reported in 2006 the average cost of Lucentis was $1,950 per injection.[102] For this reason, the off-label use of Avastin (bevacizumab), a similar drug originally designed to treat colon cancer that can be aliquoted into smaller doses for ocular injection at a much lower cost of $17 to $50 per injection, has become a popular treatment choice.[102] The Comparison of Age-Related Macular Degeneration Treatment Trials

(CATT) looked at the efficacy and safety of bevacizumab versus ranibizumab over a period of five years. The study found no statistically significant difference in VA or morphologic outcomes between the two drugs at five years. Additionally, they found no difference in outcomes whether the doctor treated with the manufacturer recommended treatment course of one injection per month over dosing pro re nata (PRN) at the doctor's discretion as determined by monthly evaluation via OCT.[103] In terms of safety, the CATT study did not find any significant difference in adverse effects, such as arteriothrombotic or venous thrombotic events, which was consistent with a larger Medicare study.[104]A study of Medicare claims for AMD treatment from 2006 to 2009 indicated that ranibizumab was chosen over bevacizumab for initial treatment only 35% of the time and, over the course of the study, the use of ranibizumab was on the decline.[105]

The latest anti-VEGF drug to hit the market is Eyelea (aflibercept, VEGF Trap-eye) which when dosed every other month has been shown to be non-inferior to monthly treatment with ranibizumab, perhaps reducing the patient burden by having less frequent treatments.[98] Therefore, all three drugs have been shown to be equally effective in initial CNV treatment.[106] Aflibercept may play a role in treating a subset of patients who initially respond well to ranibizumab or bevacizumab but after a period of time are no longer responsive to treatment.[107] Both ranibizumab and bevacizumab are antibodies, with the former being a fragmented version. However, aflibercept was designed such that multiple proteins that bind VEGF at different sites were attached to the Fc base of a human IgG giving it higher binding affinity than the previous two drugs.[108] Overall, the CATT study demonstrated that over five years, 50% of anti-VEGF treated CNV patients

maintained a VA of at least 20/40. Additionally, a Danish study found that with the advent of anti-VEGF treatments, it is estimated that the incidence of legal blindness due to AMD has decreased by 50%.[103,109]

Despite the benefits of CNV treatment with anti-VEGF, there are some downsides. There is a small risk of uveitis or endophthalmitis due to the nature of the injection, although occurrence of either of these events was found to be low at 2.3%.[90] Since VEGF is released by the body to maintain healthy tissue thickness, anti-VEGF treatment was shown to decrease retinal thickness < 120 μm in 36% of patients over 5 years. This retinal thinning has been associated with worse VA outcomes. Furthermore, anti-VEGF treatment of CNV requires multiple follow ups. During the CATT study, the average patient on a PRN dosing regimen receiving four to six injections per year with more non-injection visits in between.[103] This can be tedious for a patient, especially patients who are older with restricted mobility or whose vision inhibits them from driving.

Although anti-VEGF treatments are a major advancement in CNV treatment, they are not an easy solution for vision loss associated with CNV. Additionally, all patients do not respond to anti-VEGF treatment in the same manner. In the initial trial of ranibizumab efficacy and safety during the MARINA study, 25 to 33% of participants, depending on the dosage of ranibizumab, had an increase in VA of > 15 letters at the conclusion of the two year study.[90] This leads to the question of what predictors can clinicians use to determine who will benefit the greatest from anti-VEGF treatment? It is reasonable to predict that since AMD is multifaceted, there are many factors that could

influence treatment outcomes including age, the location and size of the initial lesion, and the time between CNV leakage and initiation of treatment. Specifically, since genetics have been shown to have an influence on AMD incidence and progression, it is logical to expect they would also have an influence on treatment. The role of genetic risk factors in AREDS treatment has already been debated with some statistical analyses indicating that the commonly prescribed AREDS vitamin supplementation increases the risk of some individuals with high CFH associated risk factors to progress to CNV AMD.[110]

Other groups have already specifically investigated the genetic role of CFH in anti-VEGF treatment outcomes. A literature search of studies to date on this topic has been summarized in Appendix A. The two largest, randomized clinical trials in the literature are the CATT and The Alternative Treatments to Inhibit VEGF in Patients with Age-Related Choroidal Neovascularization (IVAN) studies. Both of these studies indicate that there is no statistically significant difference in measured treatment outcomes based on CFH status.[111,112] However, a string of studies indicate the opposite is true with many finding that CFH status results in worse treatment outcomes and some even reporting that high risk CFH alleles results in better treatment outcomes.[113–128] The aims of this study were to investigate the role of CFH status in vision and anti-VEGF treatment outcomes in a group of patients with AMD and CNV, to add to the body of data that currently has not provided a clear answer to this question. We expect that since the Y402H CFH SNP alters the ability of CFH to bind CRP, individuals with this SNP will have reduced control over complement mediated inflammation. As a result, they will have worse

control of the inflammation associated with an anti-VEGF intravitreal injection and hence worse treatment outcomes as measured by VA.

Chapter 2 Materials and Methods

Patient recruitment, surveys, and sample collection

Patients were recruited from The Ohio State University Department of Ophthalmology. Patients were asked to participate if a chart analysis indicated they were going to be given the first of a series of three, monthly anti-VEGF intravitreal injections by retinal specialist Frederick Davidorf, M.D., for exudative AMD treatment. If there was concurrent treatment of both eyes, only the right eye was enrolled in the study. Patients were not excluded if they had received a previous series of injections. Exclusion criteria included being younger than 55 years of age and having other known causes of vision loss besides AMD. After the initial three injections, injections were given PRN. Patients were injected with bevacizumab, ranibizumab, or aflibercept at Dr. Davidorf's discretion. Once recruited, the patients were surveyed about their basic demographic information and their personal health via a modified version of the Charlson Comorbidity Index by Katz et al.[129] Patients next completed four large-print surveys: the Perceived Stress Scale (PSS), Center for Epidemiological Studies Depression Scale (CES-D), the Enhancing Recovery in Coronary Heart Disease (ERICHD) Social Support Scale, and the Impact of Visual Impairment (IVI) Scale.[130–133] Patients were given the option of having the survey read to them if they did not feel able to read the print. Visual acuity was measured in habitual correction with a back-illuminated Early Treatment Diabetic Retinopathy Study (ETDRS) chart with by-letter scoring at 2 meters. Patients were encouraged to guess, and

the stopping point was when the patient read 3 or more letters wrong on a row. Each eye was measured separately with the right eye before the left eye. If the patient used a glasses prescription, it was measured and recorded. Finally, approximately 5 mL of blood was drawn by Dr. Davidorf or another trained staff member. All data collection was completed during the patient's scheduled office visit before receiving their injection. The surveys, VA measurement, and blood draw were repeated at the time of the patient's third, and final, anti-VEGF injection of the three-part series as well as at a visit one year (+/- 1 month) from their enrollment visit resulting in a total of 3 study visits per patient enrolled (See Table 2.1).

Visit Number	Measures Documented	Time From Enrollment
1	Demographics, Comorbidity Score, VA, PSS, CES-D, ERICHD, IVI, Blood Draw	0 months First injection of three-part series
2	VA, PSS, CES-D, ERICHD, IVI, Blood Draw	3 months Third injection of three-part series
3	VA, PSS, CES-D, ERICHD, IVI, Blood Draw	1 year Follow up visit

Table 2.1 Study visit summary.

Sample processing

Blood samples were transported on ice to The Ohio State University College of Optometry. Samples were centrifuged for 20 minutes at 6°C and 1.2 RCF in a centrifuge (Eppendorf 702 R, Eppendorf, Hauppauge, NY). The plasma layer was separated into five microcentrifuge tubes with approximately 500 µL per aliquot and the buffy coat containing mostly white blood cells was siphoned off and placed in a separate microcentrifuge tube. Microcentrifuge tubes were stored immediately at -80°C (Thermo Scientific Forma 900 Series, Thermo Fisher Scientific, Carlsbad CA).

DNA purification

Total DNA was purified using the Qiagen DNeasy Blood & Tissue Purification Kit (Qiagen, Germantown, MD). The complete adapted protocol can be found in Appendix B. All DNA work was completed in a class II biological safety cabinet (Fisher Hamilton, Manitowoc, WI). The blood buffy coat sample was thawed from each patient and further aliquoted into five microcentrifuge tubes to prevent overloading the column. One aliquot was used for the DNA extraction, 2 aliquots were saved at -80°C for future studies, and the remaining two aliquots were disposed. The Qiagen protocol for Purification of Total DNA from Animal Blood or Cells – Cultured Cells was followed with the following modifications. The initial centrifugation was done at 2,100 rpm. The

volume of cells was measured using a micropipette and then PBS was added to bring the total volume to 200 µL. The final column wash with AW2 was centrifuged at 13,000 rpm instead of 14,000 rpm due to limitations of equipment available. Each column was eluted twice with 100 µL. DNA eluates were stored at -80°C. Centrifugations were completed with an Eppendorf MiniSpin and thermomixing with an Eppendorf Thermomixer R (Eppendorf, Hauppauge, NY).

SNP genotyping assay

DNA concentration was measured using an Invitrogen Quant-IT Kit and a Qubit 3.0. A Custom TaqMan SNP Genotyping Assay by Thermo Fisher Scientific was used to determine if patients had the Y402H CFP allele or not (Thermo Fisher Scientific, Carlsbad CA). The forward primer was TGTTATGGTCCTTAGGAAAATGTTATTTTCCTT and the reverse primer was GGCAGGCAACGTCTATAGATTTACC. The reporter 1 sequence was CTTTCTTCCATGATTTTG and was bound to VIC dye and the reporter 2 sequence was TTTCTTCCATAATTTTG bound to FAM dye. The Thermo Fisher Scientific TaqMan Genotyping Master Mix Protocol was followed using the 10 µL reaction from wet DNA protocol. A total of 6 ng of genomic DNA was used. The first 66 samples had completed polymerase chain reactions on a Bio-Rad CFX96 Real Time PCR machine by Anthony McCoy at The Ohio State University Plant-Microbe Genomics Facility (PMGF) (Bio-Rad, Hercules, CA). Each sample was run in duplicate and each plate contained a negative control with no sample. The PMGF closed during the timespan of the project, so

the remaining 18 samples were run on an Applied Biosystems 7900 Fast Real Time PCR machine under standard mode conditions by Paolo Fadda at The Ohio State University Genomics Shared Resource Facility (GSR) (Applied Biosystems, Foster City, CA). A sample of each of the three possible genotypes that was originally run at the PMGF was repeated at the GSR to ensure consistent results.

Data analysis

Summary statistics were used to describe patient characteristics such as age and VA at baseline. Analysis of variance and linear regression models that accounted for age, sex, smoking status, and previous injections were used to investigate the relationships among patient characteristics, SNP status, and VA. Self-reported visual functioning scores from the IVI scale were scored using Rasch analysis and logit scores were converted to a 0-100 scale for ease of interpretation. The results were analyzed using SPSS version 24 (IBM) and Winsteps version 3.69 (Linacre).

Chapter 3 Results

Participants

Eighty-four patients (51.2% female) were enrolled with a mean ± SD age of 81 ± 9 years. 100% of the patients were Caucasian. 52.4% of the patients were never smokers, 46.4% were former smokers, and 1.2%, or one participant, was a current smoker. The mean comorbidity score was 2.00 ± 1.9 at baseline. Through chart review the number of previous injections received at The Ohio State University Department of Ophthalmology was determined. At baseline, the mean number of total injections previously received by the patient in both eyes was 19.86 ± 16.4. The mean number of injections received prior to study enrollment in the treatment eye was 14.4 ± 13.0 (See Table 3.1).

Participants	84
Mean Age	81 ± 9 years
Gender	51.2% female
Smoking Status	52.4% never smoker
Mean Comorbidity Score	2.00 ± 1.9
Total Injections at Baseline	19.86 ± 16.4
Injections in Treatment Eye at Baseline	14.38 ± 13.0

Table 3.1 Description of participants.

Vision

Visual acuity was the only measure of disease severity. Mean VA of the treated eye was 0.75 ± 0.47 LogMAR at time of enrollment or 20/112 Snellen. At three months after enrollment, or study visit 2, the mean VA was 0.73 ± 0.49 LogMAR or 20/107 Snellen. At one year after enrollment, or study visit 3, the mean VA was 0.81 ± 0.49 LogMAR or 20/129 Snellen. The mean change in VA over three months was -0.02 ± 0.20 LogMAR and over one year 0.08 ± 0.23 LogMAR (See Table 3.2).

	LogMAR	Snellen
Mean VA at Baseline	0.75 ± 0.47	20/112
Mean VA at 3 Months (Visit 2)	0.73 ± 0.49	20/107
Mean VA at 1 Year (Visit 3)	0.81 ± 0.49	20/129
Mean Change in VA at 3 Months (Visit 2)	-0.02 ± 0.20	N/A
Mean Change in VA at 1 Year (Visit 3)	0.08 ± 0.23	N/A

Table 3.2 Visual acuity in LogMAR and equivalent Snellen for participants.

The VA at baseline declined with increasing age (See Figure 3.1). Visual acuity at baseline was slightly worse for former smokers than never smokers. There was only one patient who currently smoked, so the influence of current smoking on VA in our patient group could not be determined (See Figure 3.2). A higher comorbidity score, an indicator of worse overall systemic health, did not have a large influence on VA at baseline (See Figure 3.3).

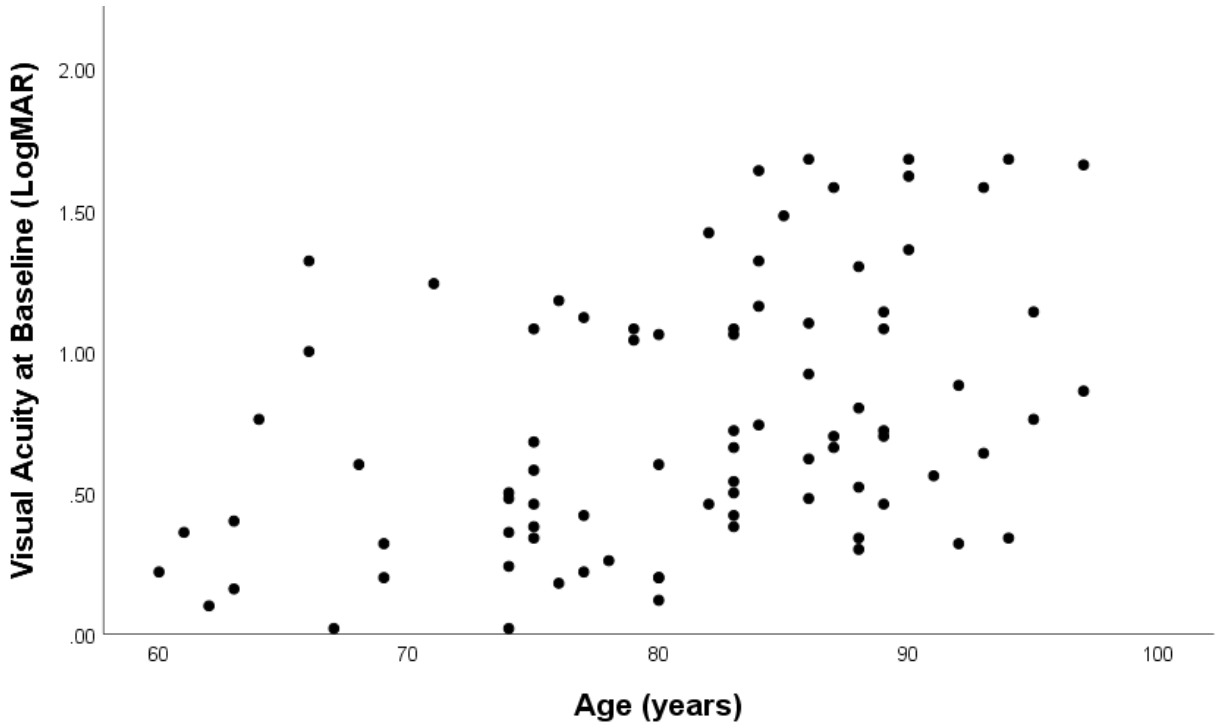

Figure 3.1 Baseline visual acuity as compared to age.

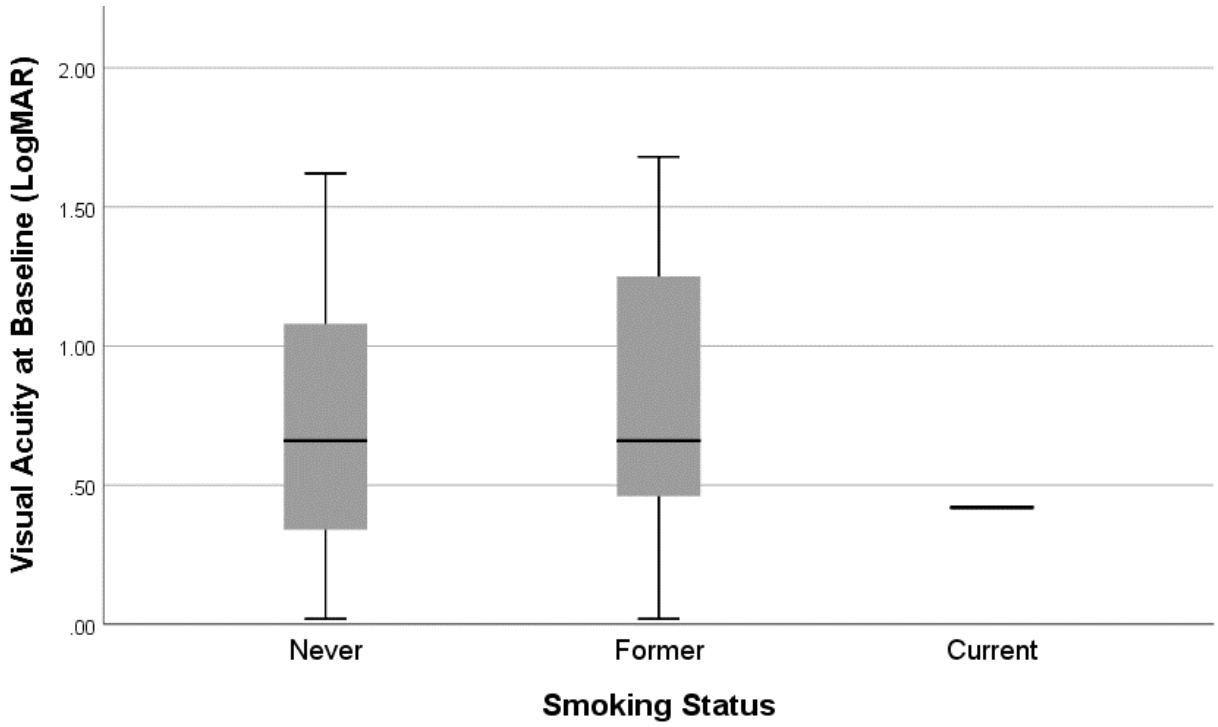

Figure 3.2 Baseline visual acuity by smoking status.

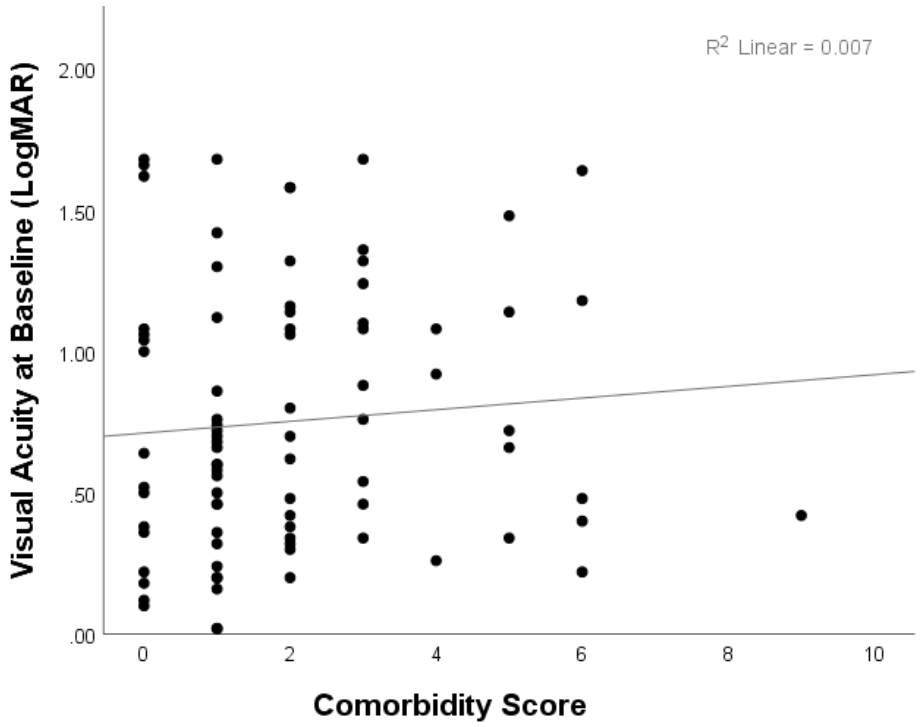

Figure 3.3 Visual acuity as compared to comorbidity score at baseline

PCR reactions

Total genomic DNA was analyzed for SNP status via quantitative PCR. Sixty-six samples were analyzed with a Bio-Rad CFX96 Real Time PCR machine. None of the reactions failed the standard quality control rules indicating a high level of reliability.[134]

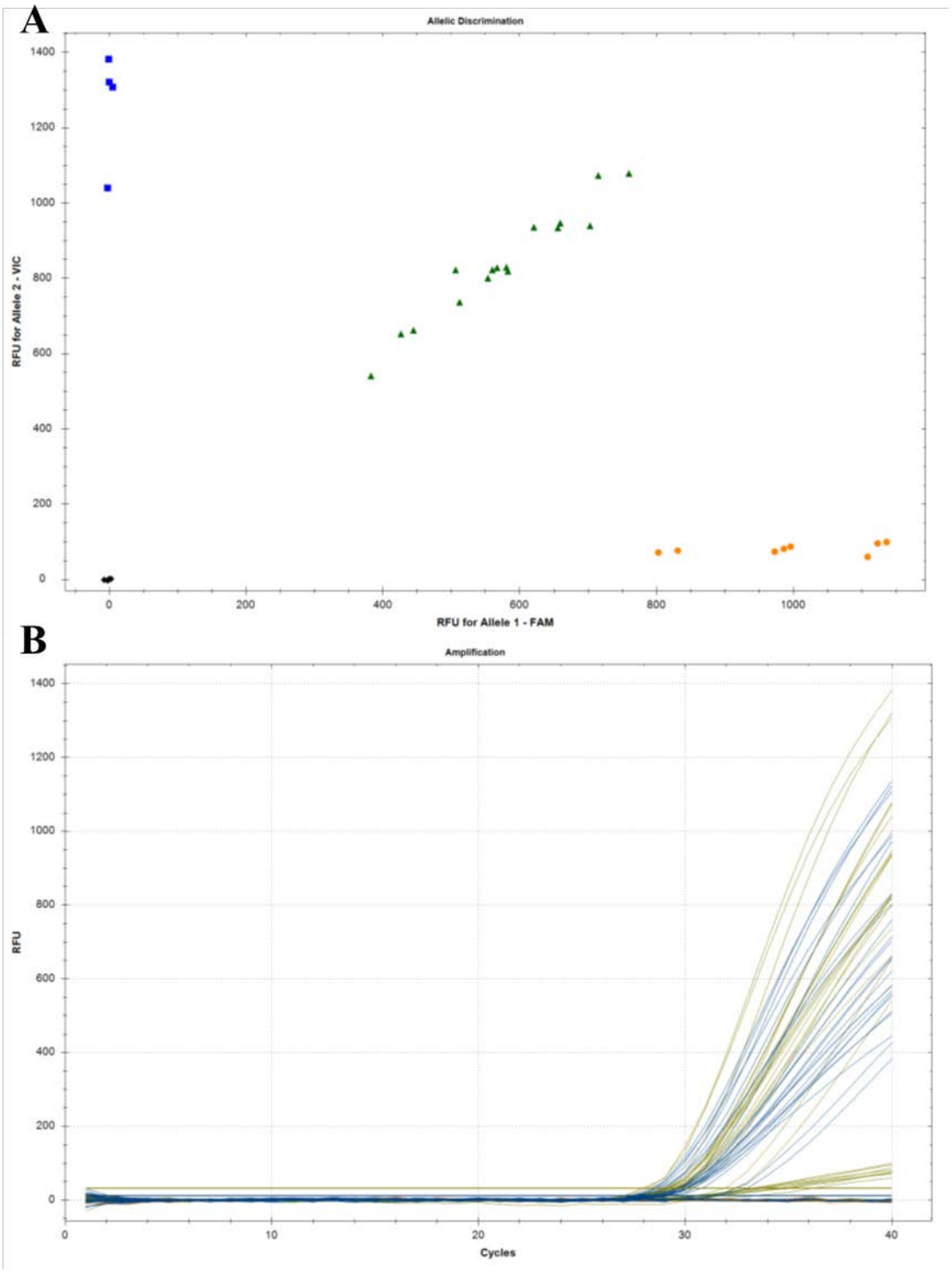

Figure 3.4 Bio-Rad CFX96 Real Time PCR results. A) allelic discrimination plot B) amplification plot with FAM in blue and VIC in green.

The remaining 18 samples were analyzed on an Applied Biosystems 7900 Fast Real Time PCR machine. All samples had a quality value greater than 99.2% indicating a high probability the assigned genotype was correct.[135]

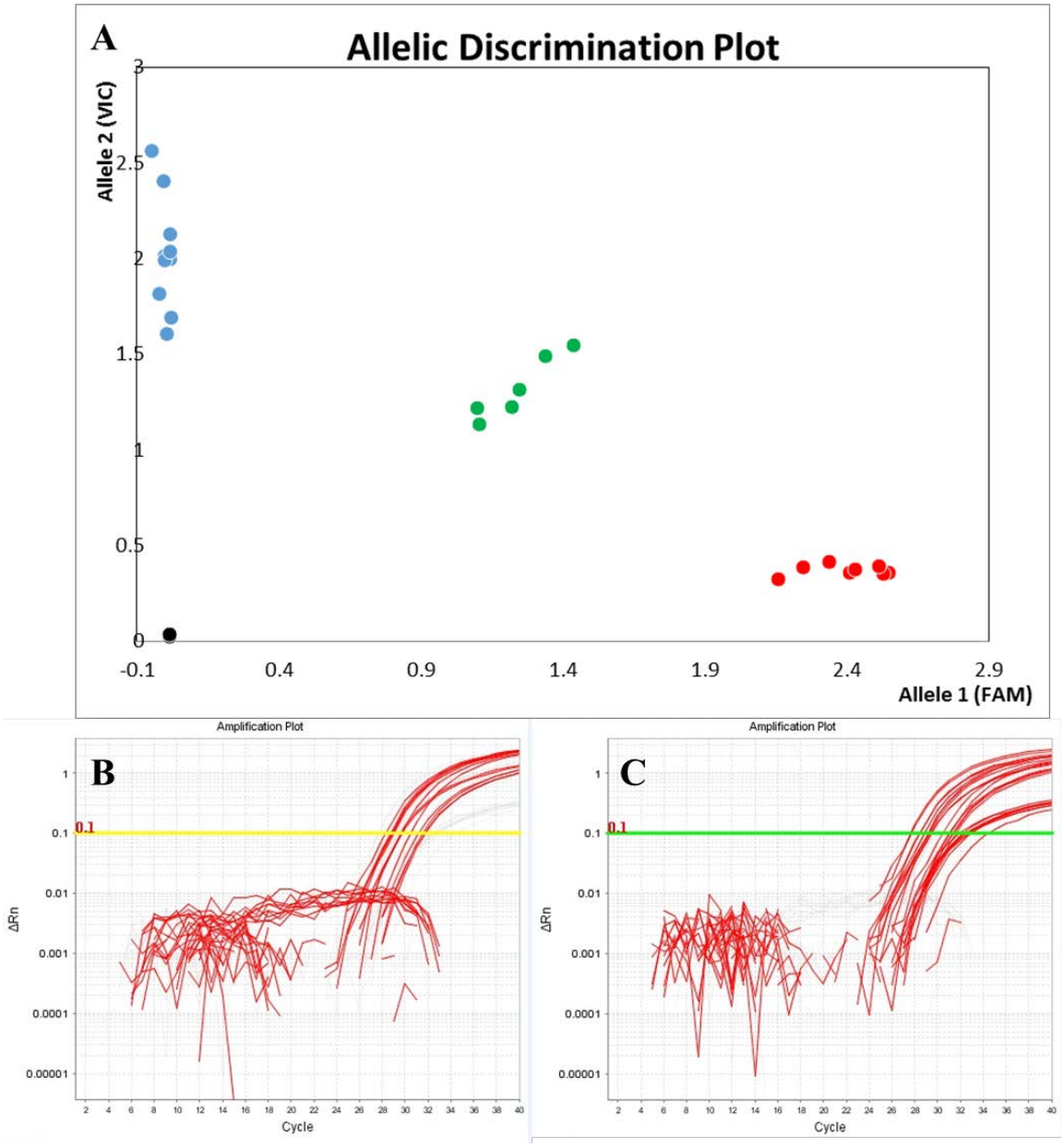

Figure 3.5 Applied Biosystems 7900 Fast Real Time PCR machine results A) Allelic discrimination B) FAM amplification plot C) VIC amplification plot.

SNP status

Of our patients, 22.6% did not have the Y402H risk factor (TT), 51.2% were heterozygous (TC), and 26.2% were homozygous (CC) (See Figure 3.6). Males and females were equally as likely to carry the Y402H risk factor (See Figure 3.7).

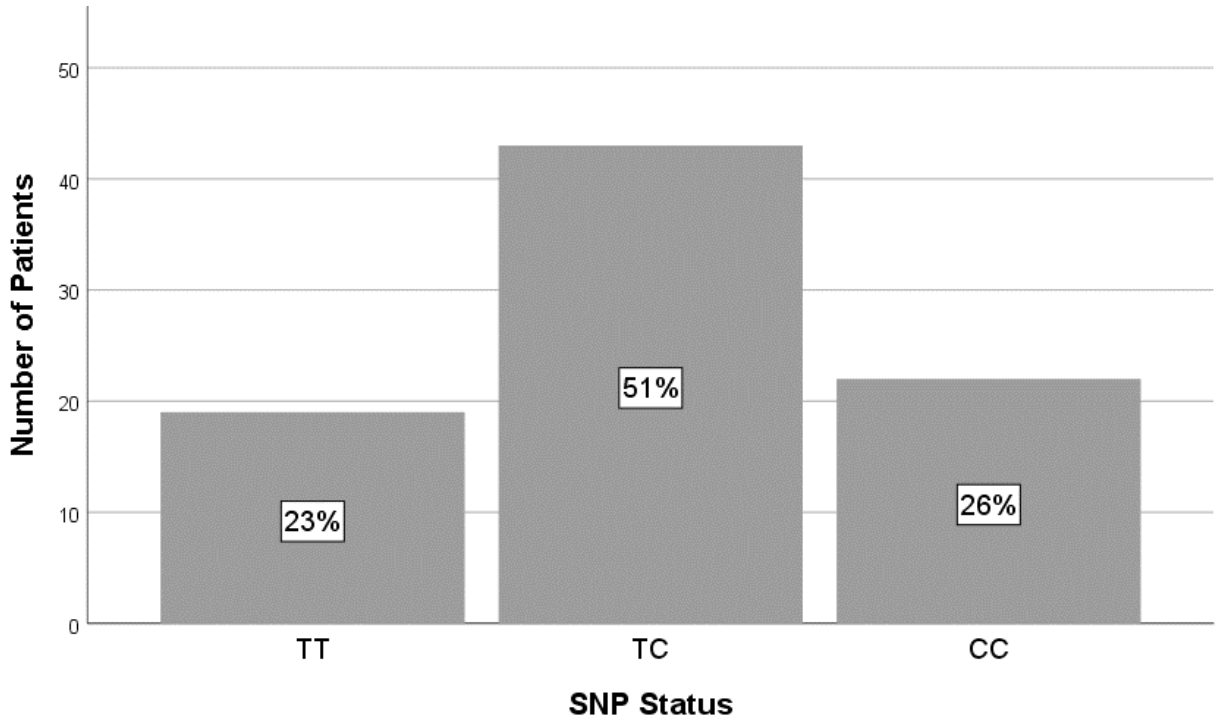

Figure 3.6 Y402H SNP status of participants. C allele is Y402H risk factor allele.

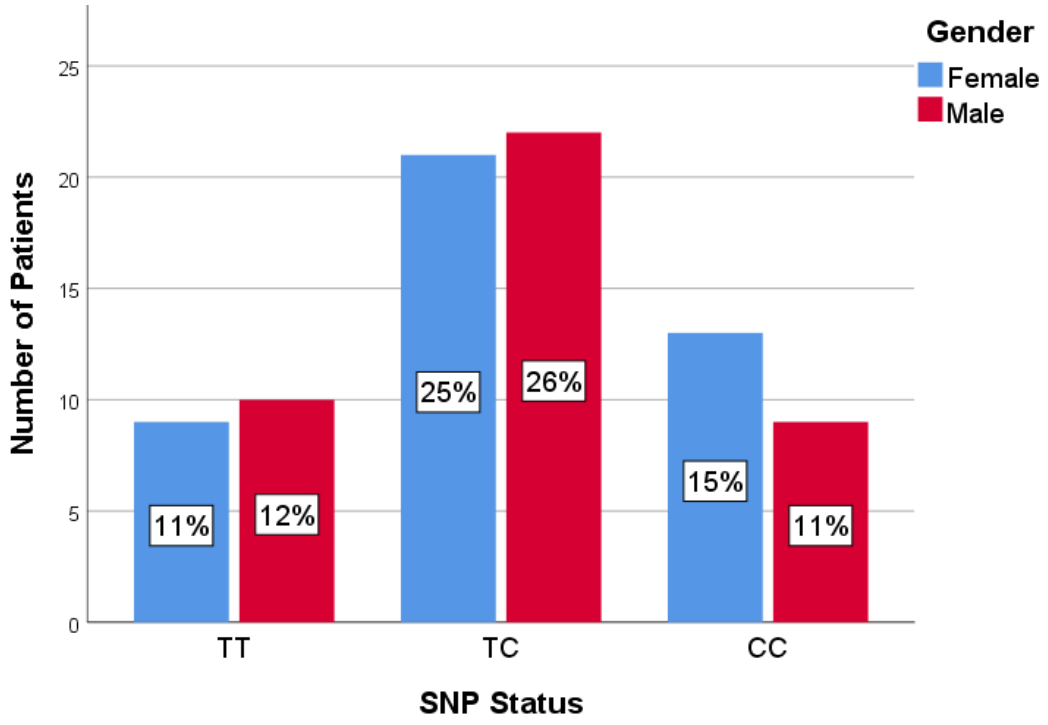

Figure 3.7 Y402H SNP status by gender.

SNP status and visual acuity

See Table 3.3 for summary of mean VA values by Y402H SNP status. A linear regression model was used to account for factors that could affect VA outcomes. Smoking status, gender, and number of injections of the treatment eye at baseline were all shown to not be predictive of VA outcomes. Visual acuity at baseline and one year was significantly associated with SNP status, with decreased VA for heterozygotes and a further VA reduction for homozygotes (See Figure 3.8) ($p = 0.018, 0.012$). There was no significant VA difference based on SNP status at three months ($p = 0.175$). Age was also

found to be predictive of VA outcome at all time points throughout the study (p = <0.001). Y402H SNP status and age were not associated with change in VA from baseline at three months or one year (See Figure 3.9). However, gender was predictive of change in VA from baseline to 1 year (p = 0.036).

	LogMAR Visual Acuity ± SD (N)		
	TT	**TC**	**CC**
Baseline (Visit 1)	0.55 ± 0.42 (19)	0.80 ± 0.49 (43)	0.84 ± 0.42 (22)
3 Months (Visit 2)	0.57 ± 0.47 (17)	0.79 ± 0.51 (39)	0.75 ± 0.46 (20)
1 Year (Visit 3)	0.59 ± 0.45 (18)	0.87 ± 0.46 (33)	0.92 ± 0.52 (19)
Change at 3 Months	-0.02 ± 0.21 (17)	0.02 ± 0.18 (39)	-0.11 ± 0.19 (20)
Change at 1 Year	0.02 ± 0.19 (11)	0.11 ± 0.22 (33)	0.10 ± 0.28 (19)

Table 3.3 Summary of mean visual acuity by Y402H SNP status.

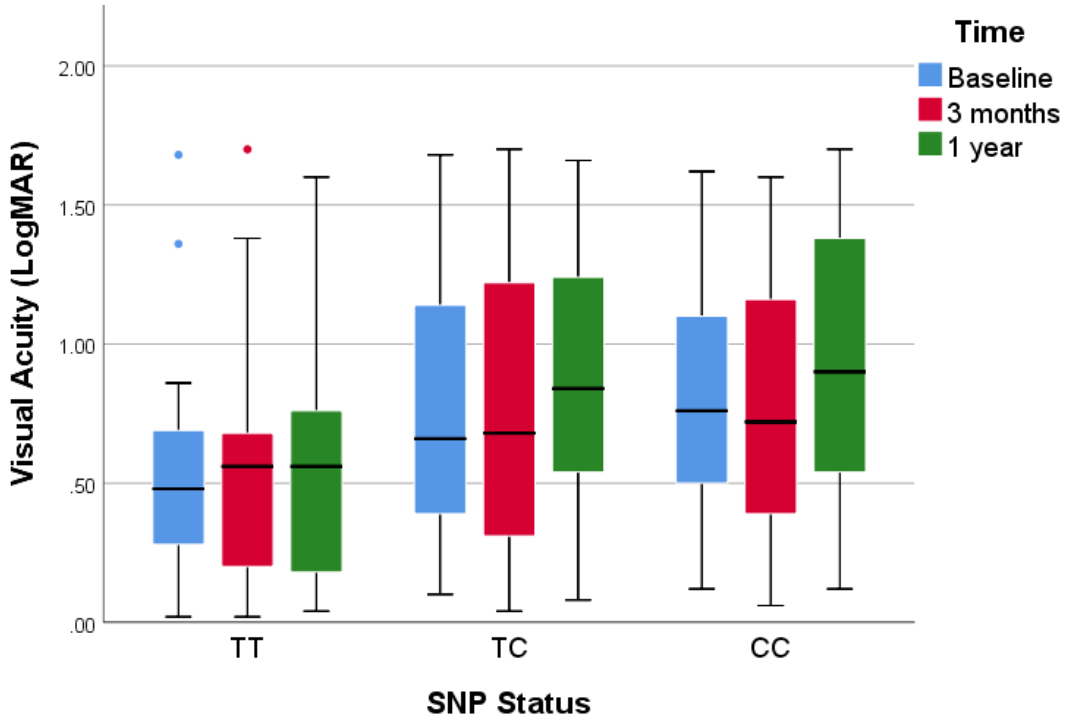

Figure 3.8 Visual acuity by Y402H SNP status at baseline (visit 1), 3 months (visit 2), and 1 year (visit 3).

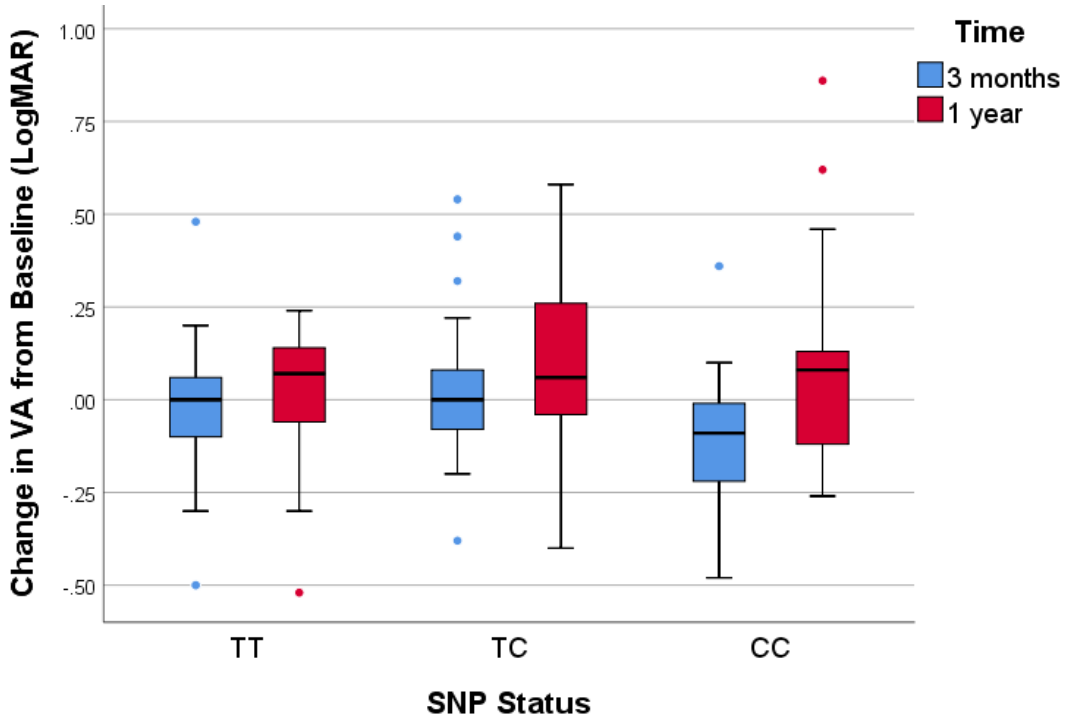

Figure 3.9 Change in visual acuity by Y402H SNP status from baseline to 3 months (visit 2) and 1 year (visit 3)

SNP status and IVI

See Table 3.4 for summary of mean IVI values by Y402H SNP status. The same linear regression analysis was used as for VA. Gender, smoking status, injections of treatment eye at baseline, and SNP status were all not predictive of IVI survey values. However, age was statistically predictive when comparing IVI values at each of the three study time points ($p = <0.001$).

	Mean IVI ± SD (N)		
	TT	**TC**	**CC**
Baseline (Visit 1)	56.65 ± 21.00 (19)	53.44 ± 18.32 (43)	48.15 ± 18.85 (22)
3 Months (Visit 2)	55.29 ± 20.73 (17)	53.15 ± 19.02 (40)	52.00 ± 20.03 (20)
1 Year (Visit 3)	56.43 ± 22.09 (18)	54.03 ± 20.65 (33)	49.38 ± 17.16 (19)
Change at 3 Months	-1.33 ± 8.68 (17)	-1.47 ± 10.51 (40)	4.07 ± 14.61 (20)
Change at 1 Year	-0.39 ± 13.11 (18)	-2.32 ± 10.63 (33)	2.14 ± 11.00 (19)

Table 3.4 Summary of mean IVI scores by Y402H SNP status.

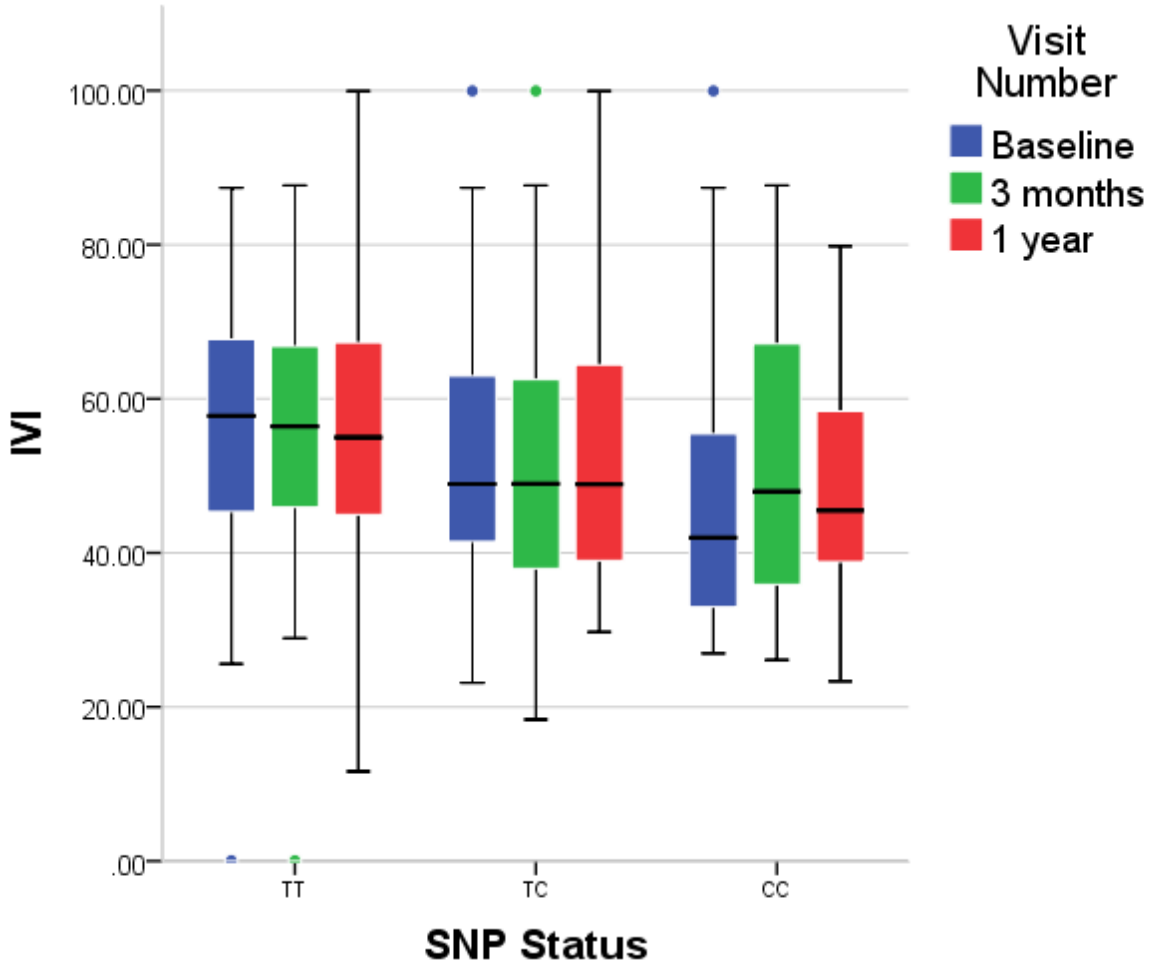

Figure 3.10 IVI scores by Y402H SNP status at baseline (visit 1), 3 months (visit 2), and 1 year (visit 3).

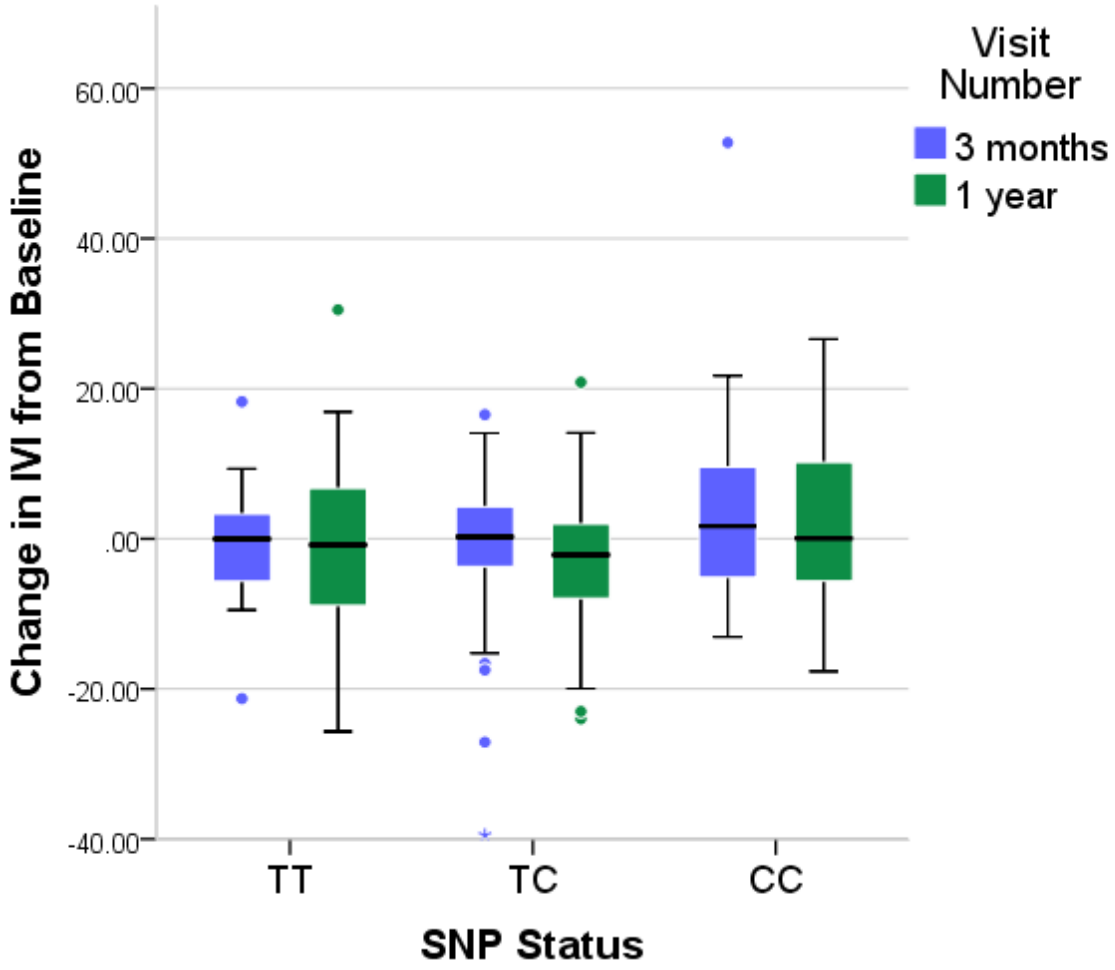

Figure 3.11 Change in IVI scores by Y402H SNP status at baseline (visit 1), 3 months (visit 2), and 1 year (visit 3).

SNP status and number of injections

A summary of the mean number of injections at baseline in total and for the treatment eye only can be found in Table 3.5. There was a significant difference in total number of injections based on Y402H SNP status (See Figure 3.12; $p = 0.034$). There

was, however, no difference in total number of injections of the treatment eye at baseline (See Figure 3.14; p = 0.124). There was no strong trend in VA at baseline with number of injections at baseline (See Figure 3.14).

	LogMAR Visual Acuity ± SD (N)		
	TT	**TC**	**CC**
Total Injections	15.05 ± 12.77 (19)	24.35 ± 18.65 (43)	15.23 ± 11.82 (22)
Total Injections of Treatment Eye	12.05 ± 11.81 (19)	17.19 ± 14.71 (43)	10.91 ± 9.15 (22)

Table 3.5 Summary of number of injections at baseline by Y402H SNP status.

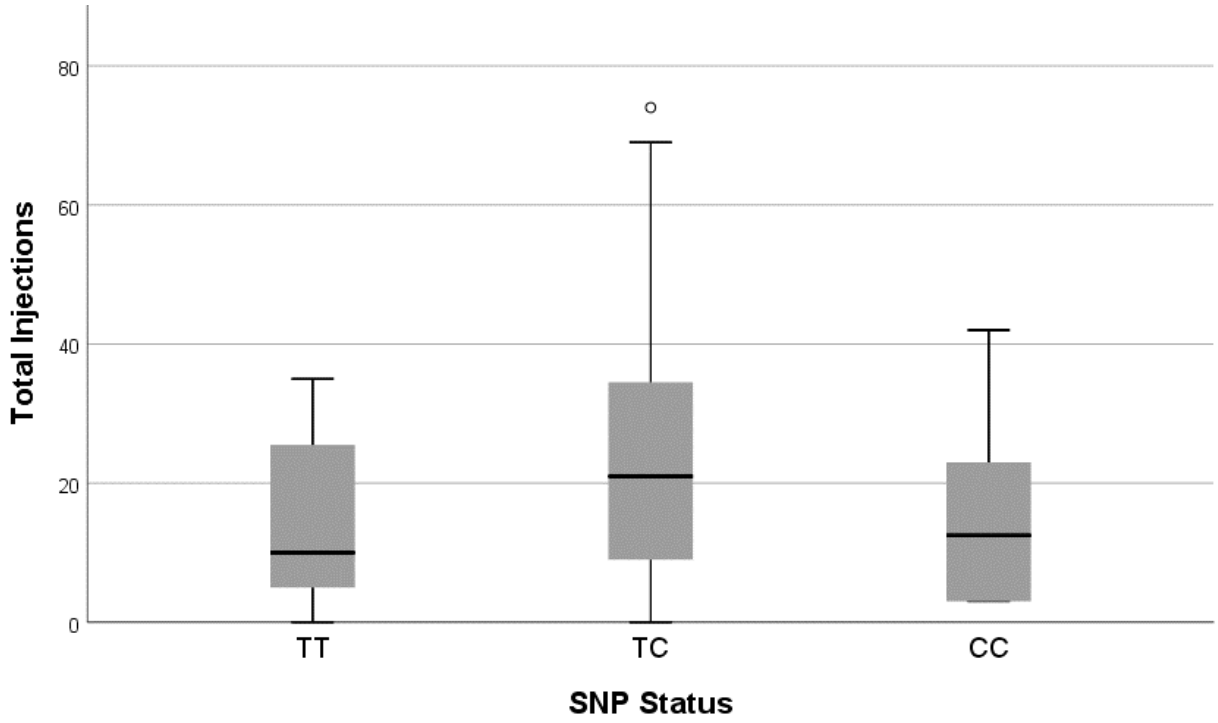

Figure 3.12 Total injections at baseline by Y402H SNP status.

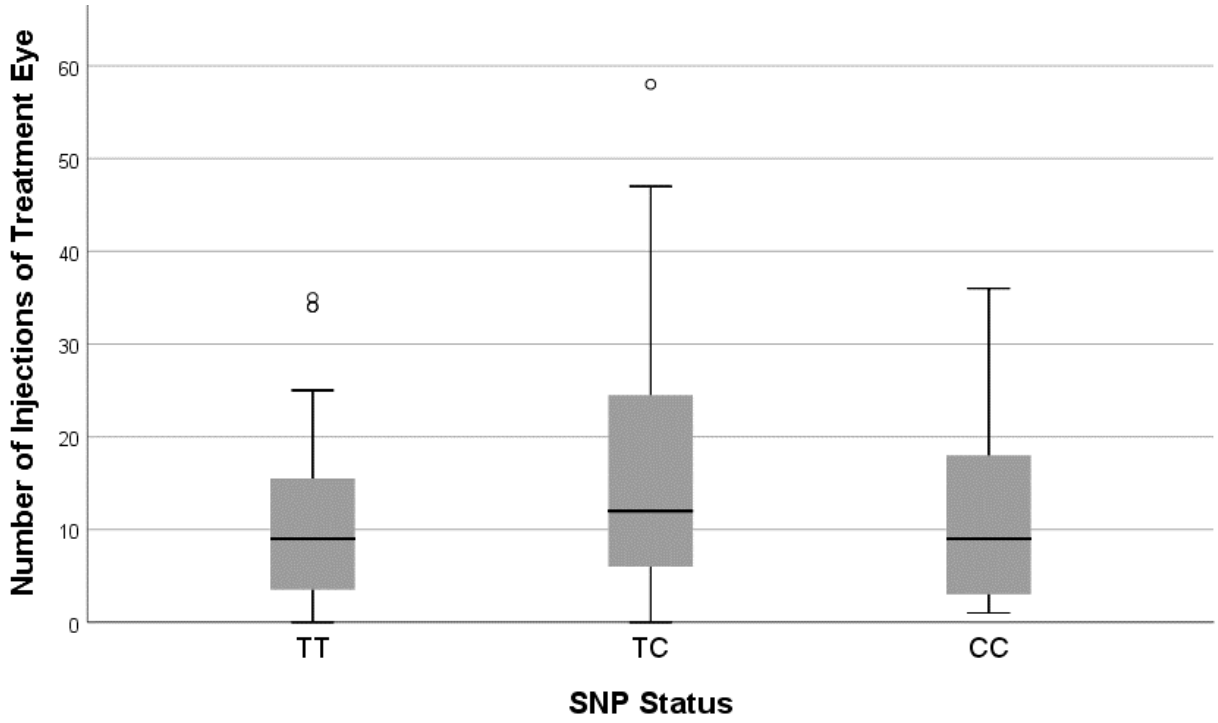

Figure 3.13 Number of injections of treatment eye at baseline by Y402H SNP status.

Figure 3.14 Number of injections of treatment eye by visual acuity at baseline.

Chapter 4 Discussion

Our data add to the growing body of literature that evaluates the role CFH Y402H plays in AMD prognosis and treatment. As of September 2018, there have been thirty-four published papers that investigate the influence Y402H status has on CNV treatment outcomes with anti-VEGF.[111–128,136–151] A summary of these studies and their major outcomes can be found in Appendix A and are discussed in further detail in relation to this work here.

Frequency of the C allele

Of those thirty-four studies, twelve reported primarily Caucasian or white patients like the demographics of our study. For those studies that reported a breakdown of the patients with the TT, TC, or CC alleles there was a trend that is consistent with our data. The heterozygous portion makes up approximately 50% (45.5-66.3%) of the group and those homozygous for the CC risk factor (22.1-35%) is, on average, larger than the TT allele. [112–114,122,126,136,141,144,147] The exception was McKibbin et al. in which both homozygous genotypes were approximately equal (See Figure 4.1).[119] Similar proportions to those found in Caucasian populations were noted in Turkish, Tunisian, and Brazilian populations although the TT and CC genotypes have been reported to be more equally distributed in Brazilian populations.[118,127,128,143,150] One Turkish study found

results more similar to that of Asian populations including Japanese, Chinese, Malaysian, and Korean where the CC genotype was rare (0-6%) and the TT genotype predominated.[121,137,139,140,146,148,149,151] A confounding factor in Asian populations is the higher prevalence of polypoidal choroidal vasculopathy (PCV), which often causes subretinal bleeding that can be hard to distinguish from choroidal neovascular AMD. Matsumiya et al. specifically made a point to treat neovascular AMD patients who had concurrent PCV and found that PCV reduced resolution of the neovascular AMD lesions in patients who had aspects of both diseases.[148]

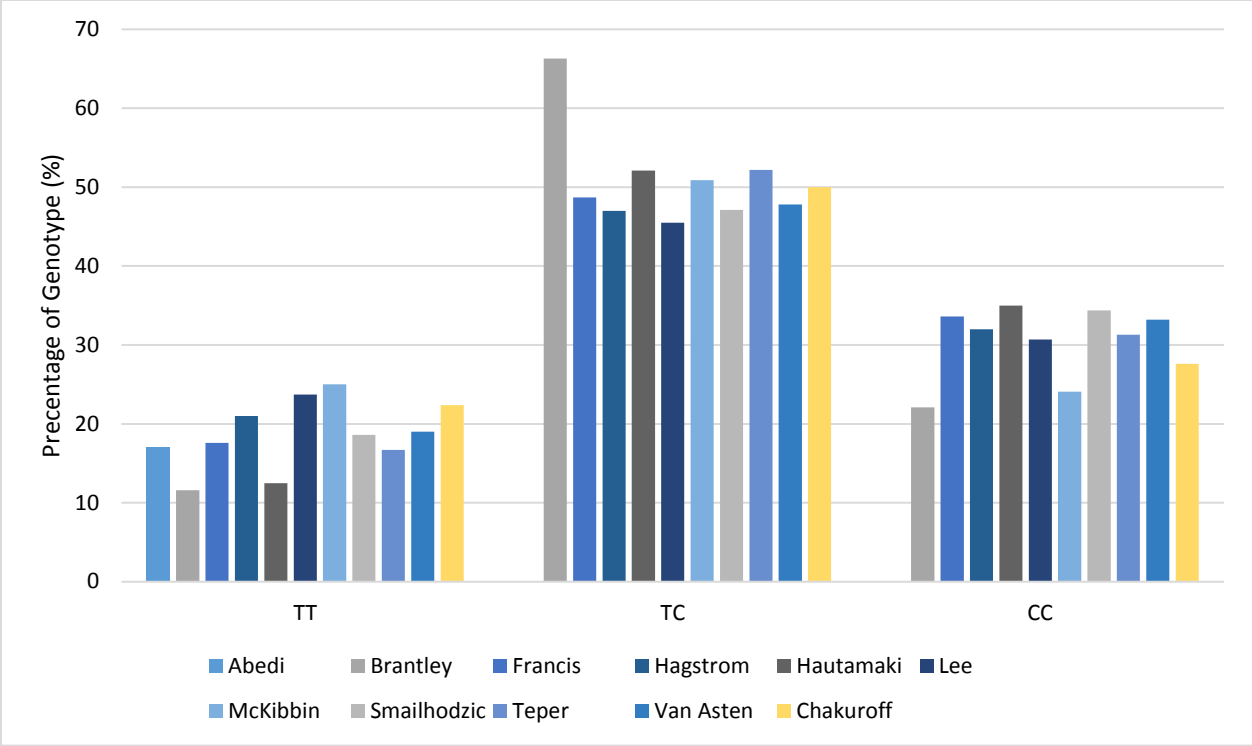

Figure 4.1 Comparison of Y402H SNP genotype rates in Caucasian population. [112–114,119,122,126,136,141,144,147]

The average C allele frequency found by these ten Caucasian studies of neovascular AMD patients seeking anti-VEGF treatment was 56.9%.[78,82,112–114,119,122,126,136,141,144,147] This is notably higher than the 36% frequency of the allele in the general Caucasian population. This allele is also more common in those who progress to neovascular AMD, a late-stage of the disease, than those with early or intermediate stages.[81,83] We can conclude that at least for the Caucasian population, having one or two copies of the C allele increases the risk for one of the more advanced stages of AMD and the likelihood of having associated vision loss or requiring anti-VEGF treatment. This

supports the argument for including genetic testing as part of the standard of care for patients with early signs of AMD. However, we know there are multiple genetic risk factors beyond CFH Y402H that can increase or decrease the risk of developing AMD. Although there is substantial data known about CFH Y402H, its role in the greater picture of how it interacts with other genetic and environmental factors still leaves the value of genetic testing up for debate. For example, the most widely known in office genetic test for AMD, Macula Risk, assesses 15 SNPs in patient samples. Although it includes a CFH SNP, their predictive algorithm chooses to neglect the Y402H SNP (rs1061170) despite it being the most well-studied and understood genetic risk factor for AMD.[152]

Visual acuity outcomes with the C allele

Of the thirty-four studies that evaluated treatment outcomes of anti-VEGF based on Y402H SNP status there are contradictory results. The studies are listed in Table 4.1 based on whether their results support Y402H SNP status affecting VA outcomes or not. Two studies only looked at anatomical changes on OCT as a measure of treatment outcomes. [111,144] Both showed that Y402H SNP status had no effect on VA outcome. Cobos et al. looked at anatomical changes and VA changes, but only found SNP status to have an effect on anatomical outcomes as measured by central foveal thickness.[153] It is included in the table under SNP status having an effect for this reason.

No Y402H SNP Effect	Y402H SNP Effect	
	CC Worse Outcome	*CC Better Outcome*
Francis et al. 2011*[136]	Brantley et al. 2007*[113]	McKibbin et al. 2012*[119]
Kang et al. 2012[137]	Lee et al. 2009*[114]	**Cobos et al. 2017***[153]
Orlin et al. 2012[138]	Imai et al. 2010[121]	
Tian et al. 2012*[139]	Teper et al. 2010[122]	
Yamashiro et al. 2012[140]	**Kloeckener-Gruissem et al. 2011)**[123]	
Abedi et al. 2013*[141]	Nischler et al. 2011*[124]	
Chang et al. 2013*[142]	**Menghini et al. 2012***[125]	
Habibi et al. 2013[143]	**Smailhodzic et al. 2012***[126]	
Hagstrom et al. 2013*[112]	Dikmetas et al. 2013[127]	
Hautamäki et al. 2013*[144]	Veloso et al. 2014*[128]	
Kitchens et al. 2013*[145]	Beykin et al. 2015*[115]	
Lotery et al. 2013*[111]	Piermarocchi et al. 2015*[116]	
Park et al. 2014*[146]	Shah et al. 2016[117]	
Van Asten et al.2014*[147]	Sengul et al. 2018[118]	
Matsumiya et al. 2014*[148]		
Kepez et al. 2016[149]		
Medina et al. 2016[150]		
Mohamad et al. 2018*[151]		

Table 4.1 Significant difference in visual acuity outcomes on Y402H SNP status study summary table Studies in bold have n > 200. Studies with asterisk included only treatment-naïve patients. Studies in red did not report visual acuity as an outcome, but instead looked at anatomical changes on OCT.

Eighteen groups found there was no difference in VA based on SNP status.[111,112,136–143,145–151] Included in this list are seven studies that reported none, rare, or less than 7% CC genotypes and an additional study on a Korean population, expected to have low rates of C allele in their population but did not report the allele frequency in their publication.[137,139,140,142,146,148,149,151] Because the occurrence of the C allele was so low in

these populations, these studies may not have sufficient power in describing the affect the C allele had on VA outcomes. This group also included the results from the largest study performed on this topic by Hagstrom et al. This work was a prospective study of 834 patients at 43 clinical centers across the United States as part of the Comparison of Age-related Macular Degeneration Treatment Trial (CATT). The CATT Research Group has evaluated the difference in treatment outcomes over five years between ranibizumab (Lucentis) and bevacizumab (Avastin).[103] All patients were treatment-naïve at time of enrollment, eliminating previous treatment as a confounding factor. The study found no statistically significant differences in VA outcomes, anatomic outcomes, or number of injections required by patients on a PRN treatment regimen between Y402H SNP status genotypes. They also evaluated a SNP in ARMS2, HTRA1, and C3 and found the same results. Additionally, the study found no difference in response to treatment based on the total number of risk alleles an individual had (up to eight risk alleles as four SNPs were tested).[112] The studies that demonstrated no difference also includes data from two other major prospective studies on treatment-naïve patients. First, the Alternative Treatments to Inhibit VEGF in Patients with Age-Related Choroidal Neovascularization (IVAN) trial based in the United Kingdom with 254 participants tested 484 SNPs and found only one in the HTRA1/ARMS2 that showed a slight association with nonresponse, as defined by change in total retinal thickness from baseline.[111] The second study evaluated 391 patients in the European Genetic Database (EUGENDA) study. This group is based out of Germany and The Netherlands and continues to complete research that focuses on the genetic aspects of AMD. They found nonresponse, as defined by losing greater than or

equal to 30% of letters at baseline, was not associated with any of the eight SNPs evaluated. In contrast to Hagstrom et al. the EUGENDA study did find that the total number of cumulative risk alleles was an independent predictor of nonresponse.[147] These three major, well executed studies demonstrate that CFH Y402H SNP status does not predict worse outcomes with anti-VEGF treatment. Our data agree with these results.

There are, however, sixteen studies that demonstrate the results contradictory to ours. Of these studies that indicate Y402H SNP has an effect on treatment outcomes, there is only one, of a Japanese population, that reported no occurrence of the CC genotype compared to the eight studies with low occurrence of the C allele found in the group of publications that demonstrated no difference in VA based on SNP status.[121] This collection of studies showing an effect of Y402H contains no large, prospective research trials. The largest study was conducted in association with the EUGENDA study, in which 420 eyes of 397 patients were enrolled. Patients were treated in Germany, The Netherlands, and Canada. The data for 347 patients was collected via retrospective chart review and 78 patients were followed prospectively with a VA measurement protocol not used with the retrospective VA measurements. They found that low risk TT patients performed better by an average of 5.3 letters on ETDRS VA than those with high risk CC genotype. They also found a cumulative effect when CFH Y402H risk alleles were present in conjunction with an ARMS2 risk allele.[126]

Two studies demonstrated that the Y402H high risk allele actually improved treatment outcomes. The first by McKibbin et al. showed an improvement in VA at 6 months that was greater in CC patients than TT or TC patients.[119] The second study by

Cobos et al. found that after the initial loading phase of three injections (three months) CC patients had a more dramatic reduction in central foveal thickness (CFT) on OCT than TT or TC patients.[120] Although both of these studies demonstrate the opposite response of what is reported in other studies, it is most likely because both of these results are reporting on initial changes. If the C allele causes worse progression of the disease, and therefore potentially a worse starting point at time of treatment, there is more room for improvement to be shown than a milder case of CNV. However, this response was not consistent with other studies that looked at initial response in treatment naïve patients. A better understanding of these results could be found by comparing these two studies specifically to the 3-month time points of all studies on treatment naïve individuals.

Our data are in alignment with studies supporting the hypothesis that Y402H SNP status does not affect visual outcomes after anti-VEGF treatment. We found no statistically significant difference in VA changes based on Y402H SNP status. However, since our patients were not treatment-naïve and had an average of 14.4 injections of their treatment eye prior to enrolling in our study, some conclusions from the baseline data can be made. At the time of enrollment, the patients with CC genotype had significantly worse VA than those with TC or TT and, TC worse than TT. Our patients with the C risk allele also had statistically significant worse VA at the one-year time point in our study. Although we did not see a trend in VA changes within our one-year study, it should be noted that the majority of our patients had been receiving regular anti-VEGF treatment prior to enrollment. Whether the worse VA at baseline and 1 year in patients with the C

allele is attributable to a more progressive form of the disease with worse vision outcomes or a worse response to their treatment cannot be determined using these data.

When looking at all thirty-four studies that previously evaluated the association between Y402H SNP status and anti-VEGF treatment outcomes, it is somewhat perplexing to not find a common answer to how effective anti-VEGF is in patients who carry the Y402H SNP. The most likely explanation is due to variability in study design. Some studies were only conducted on treatment-naïve patients while others included patients with previous treatments. Some studies looked at changes over a short time frame such as a few months, while others looked at changes over a year. The definitions of a "good response" varied and included loss or gain of a number of lines, number of letters, or percentage of letters from baseline on either Snellen VA or ETDRS charts. Even if the method of VA measurement was consistent, some studies performed refraction and found a best corrected visual acuity (BCVA) at each study visit while others, including our study, measured with habitual correction. Statistical analysis typically fell into one of two methods, comparing between the three possible genotypes at this SNP (TT, TC, and CC) or comparing responders vs. non-responders based on that study group's interpretation of a good response and then looking for genetic differences between the two groups. Dikmetas et al. eliminated all patients with no change in VA from their statistical analysis, and Lotery et al. excluded the middle 50th percentile of their patients from their analysis by classifying responders as the top 25th percentile of VA change and non-responders as the bottom 25th percentile of VA change.[111,127] With all these small differences in study design, it can be difficult to parse out exactly what is

causing the variable results investigators encounter when they attempt to answer what role Y402H plays in anti-VEGF treatment outcomes.

In an attempt to look at the questions on a larger scale, there have been three meta-analysis on parts of the total data set. The first was by Han Chen et al. which evaluated six studies and 808 patients and found the CC genotype to be a predictor of poor response to anti-VEGF and photodynamic therapy treatment (PDT), and that Y402H status was a better predictor of poor response for anti-VEGF treatment than for PDT.[154] The second meta-analysis was conducted by Guohai, Chen, et al. This group looked at 13 studies with a total of 2,704 patients. They found that the CC genotype was a predictor of worse outcomes when compared to either TT or TC. They also completed a sub-analysis of the studies that only used change in VA as an outcome measure. This consisted of 8 studies and 1,903 patients. The sub-analysis showed that when VA was evaluated alone with no change in morphology (resolution of macular edema) being included in the definition of response, there was a stronger effect of Y402H status on treatment outcome.[155] The last meta-analysis by Hong et al. included 14 studies and 2,963 treatment-naïve patients. They also found that patients with the CC genotype had worse treatment outcomes than TT, but found no difference between CC+TC versus TT indicating no significantly worse outcome with just one risk allele. However, when a sub-analysis was run using only the Caucasian studies (n=10), a statistically significant difference with both CC and TC was identified.[156]

Our study is the first, to our knowledge, that not only measured VA as a treatment outcome, but also evaluated the patient's subjective perception of their vision through the

IVI Scale. This full 32-item validated scale asks questions in five domains of function: leisure and work, consumer and social interaction, household and personal care, mobility, and emotion reaction to vision loss.[157] Based on the subscale Rasch analyses of Lamoureux et al. three different domains were defined: mobility and independence, emotional well-being, and reading and accessing information. Since we evaluated emotional well-being and independence with the PSS and CES-D, we only administered the nine item subscale for reading and accessing information instead of the full 32 item survey (See Appendix C).[1] Although other studies report changes in VA or OCT, perhaps the most useful treatment outcome measure is how vision loss from AMD alters the patient's interaction with the world as indicated by the IVI. Our IVI results indicate that there is no statistically significant difference based on Y402H SNP status in IVI values at any of our study points or in any change in IVI values over time.

Other factors on visual acuity

In a study on the natural progression of neovascular AMD in 4362 patients, the average age of the patients was 74 years with 57.5% female. Our study group was older with an average age of 81, but similarly matched in gender compared to other neovascular AMD patients. The average VA of neovascular AMD patients was slightly better at 0.64 logMAR or 20/87 Snellen compared to our patients' average VA at baseline of 0.75 logMAR or 20/112 Snellen.[158] The slightly worse VA could be because our patients were older or because most of our patients were not treatment-naïve. The mean comorbidity score of our patients was 2 which matches the mean for patients 80 to 89

years old, as determined by the Charlson Comorbidity Score validation studies.[159] This indicates that our patients were generally as healthy as other older populations.

Although Y402H SNP status was not predictive of changes in VA during our study, gender was found to be predictive in VA change from baseline to 1 year. This could possibly have to do with one gender being more prone to regular follow up with their doctor throughout the course of the study. Our female and male patients were similar in age with a mean female age of 81 and mean male age of 82 indicating age was likely not a factor in this difference.

Finally, there was a statistically significant difference in number of injections at baseline based on SNP status with the heterozygous TC allele group having the most injections. Again, age was similar in each of the possible three Y402H SNP alleles with an average of 82 years for TT, and 81 years for TC and CC, as such, having lived longer and having more time for previous injections is not a probable explanation for this finding.

Limitations of this study

One of the limitations of our study is that not all of our patients were treatment-naïve. Only six percent of our patients had not received previous injections and the average number of injections at time of enrollment was 14.1. Comparing treatment-naïve to non-treatment-naïve patients is not ideal as the initial response of a lesion to treatment, as found by the MARINA study, was greatest at 3 months after treatment initiation.[90] Many similar studies on the topic chose a 3 month period of observation for their study

based on this result. One study by Van Asten et al. specifically compared response at 3 months to response at 12 months and found that non response at 3 months was a significant predictor of non-response to treatment at 12 months.[147] Additionally, patients enrolled in our study may have been beginning a series of injections for a new lesion or starting a second series of treatment for a lesion that had been previously treated. This may have altered the response of the lesion. Another limitation is that not all of our patients had the same number of injections during the year of the study. Each patient began with three injections, once a month for three months, but subsequently, injections were given PRN. However, the CATT study identified no difference in outcomes between monthly and PRN treatment.[112]

In our study we did not take into account the size or location of the lesion being treated. We also did not note whether the lesions were largely occult or classic choroidal neovascularization. Van Asten et al. evaluated the effect of lesion type and size on treatment outcomes and did not find a difference.[147] However, it seems likely that a central lesion would have worse VA at baseline than a paramacular lesion. It would have been useful to have OCT and fundus photography of treated lesions to evaluate where lesions were located originally, and, as they resolve, what regions of the retina were most affected. Future studies might include retrospective analysis of chart data to determine some of this information to better answer what role lesion size, location, and type might play.

Finally, we did not measure BCVA at each study visit; only vision in the patient's habitual correction was measured. It is possible that upon enrollment in our study the

patient was not wearing the proper correction for their refractive error or that during the treatment process a change in central retinal thickness could cause a change in refractive error. An analysis of a subset (n=74) of our patients was completed as part of a different project. This analysis compared VA with habitual correction and autorefraction at time of visit. The majority of subjects did not have improved VA with autorefraction, and the average spherical equivalent difference between habitual correction and autorefraction in those who improved was relatively small (0.54 diopters).

In our study, ranibizumab, bevacizumab, and aflibercept were all used for treatment based on the judgement of Dr. Davidorf, though the vast majority received bevacizumab injections. Ranibizumab and bevacizumab have been shown to be equally effective over 5 years in the CATT study and 2 years in the IVAN study.[95,103] Furthermore, aflibercept has been shown to be equally as effective as ranibizumab.[98] However, aflibercept has shown the potential to be more effective in patients who have lesions that are resistant to bevacizumab or ranibizumab.[107] It is thought that a small percentage of patients on long term anti-VEGF treatment experience tachyphylaxis in response to bevacizumab and ranibizumab and switching to the other treatment resulted in an improvement in 81% of patients.[160] Aflibercept has been shown *in vitro* to have a higher binding affinity to VEGF than bevacizumab and ranibizumab which could explain how, in some scenarios of long term treatment, it is more effective.[107,161]

Future studies

As it is still not clear what factors can be used to predict a good or poor response to anti-VEGF treatment, there is a need for additional studies. CFH Y402H is just one of many genetic risk factors that have been shown to play a role in AMD incidence or progression (See Table 1.1). More studies of CFH Y402H in conjunction with other risk factors could help better elucidate the role of genetics in treatment outcomes. There are also other genes that have a protective function in AMD and others that dramatically increase risk but are very rare. It is difficult to determine which genes are most important to study to have the greatest and most feasible potential impact on how we treat the disease. In a recent review of genetics and AMD, Warwick and Lotery state that approximately 50% of AMD cases can be attributed to hereditary factors.[57] However, this means that there is still a large amount of influence of environmental risk factors such as age, smoking status, and overall health. For this reason, it is hard to generate a genetic risk test with widespread use and, in fact, previous attempts have shown that risk models that include genetic components are only marginally more predictive than models that only account for environmental risk factors.[57] Furthermore, using genetics to predict response to the primary treatment of most forms of AMD, AREDS vitamin supplementation, has been wrought with controversy after analysis by Awh et al. of AREDS data indicated that certain CFH SNP genotypes, not Y402H, have an adverse response to zinc supplementation that was not present in the analysis of the original AREDS investigation.[162] For these reasons, at this time it is still the recommendation of

the American Academy of Ophthalmology to not routinely obtain genetic testing in AMD management.

Another area of interest is how stress and depression alter the different Y402H genotype responses to anti-VEGF. Acute stress and depression have been shown in many studies to elevate CRP in the body.[163,164] C reactive protein has long been studied as a marker of inflammation and a potential predictor of disease. C reactive protein makes for a good measure of inflammation since it is not affected by circadian rhythms, food intake, or gender and is structurally stable if frozen and thawed.[165] Over 30 studies have shown that CRP levels above 3 mg/L puts an individual at "high risk" for future inflammatory based cardiovascular events like myocardial infarction, stroke, and peripheral arterial disease.[165,166] Perhaps most significantly, in those with elevated CRP levels (>3mg/L) there was a significantly increased risk of AMD.[167] In particular, high CRP levels are associated with late AMD, as opposed to early stages.[74,168]

We already know that the Y402H portion of CFH is responsible for binding CRP.[66] Since we see that individually both elevated CRP and the CFH Y402H polymorphism results in an increased risk of AMD, the question arises if these two factors interact.[73] Since CRP levels are a potentially modifiable risk factor, knowing how the protein interacts with non-modifiable genetic risk factors could help reduce the increased genetic risks. One study by Despriet et al. demonstrated that stimulators of the complement cascade such as smoking, elevated ESR, and elevated serum CRP levels in those homozygous for the Y402H polymorphism increased their risk for AMD.[82] This group also took into account CRP haplotypes and found three different variations, two of

which, when in concert with the Y402H polymorphism, resulted in a higher risk of AMD, although the haplotypes did not pose any increased risk on their own.[82] Another analysis has shown a synergistic, super-additive increase in risk for late AMD prevalence and AMD progression in those with CRP over 5 mg/L and the CC genotype. This statistical relationship indicates some biological interaction between CRP and CHF.[74]

Currently, our research group is analyzing the CRP levels of patients enrolled in the study. It would be interesting to know if our patient CRP levels increase due to the stress of receiving regular injections or to depression associated with AMD and long-term struggles with vision loss. Our patients may also have elevated CRP levels due to other inflammatory diseases such as atherosclerosis and associated myocardial infarction and stroke. If elevated CRP levels normally signal through CFH inhibition of the complement cascade, it is reasonable to think that those with abnormal CFH at the Y402H position would have worse regulation of these inflammatory pathways and perhaps worse treatment outcomes after anti-VEGF injection.

Conclusion

Our data support the conclusion that there is no effect of Y402H SNP status on change in VA during treatment with anti-VEGF injections. However, our baseline data indicates that those with the Y402H risk factor have worse VA. Since many of our patients at baseline had already received anti-VEGF treatment, this data supports the idea that those with the Y402H risk factor have worse treatment outcomes, or at least more severe forms of the disease that are more resistant to treatment. Extension of this work

will evaluate the data presented here in conjunction with other measured factors such as CRP levels, stress, depression, and overall indicators of health to better understand what factors can be modulated to best promote effective treatment outcomes for patients with exudative AMD.

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Appendix A. Literature Review Summary Table

A PubMed literature search with the criteria "complement factor h" or "CFH" or "rs1061170" and "macular degeneration" or "AMD" and "vascular endothelial growth factor" or "VEGF" or "anti-VEGF" or "bevacizumab" or "ranibizumab" or "aflibercept" as adapted from Hong et al. was completed on September 15th, 2018.[156] Abbreviations in the table are defines as follows: NR = not reported. ETDRS = Early Treatment Diabetic Retinopathy Study. BCVA = best corrected visual acuity. OCT = optical coherence tomography. CRT = central retinal thickness. CFT = central foveal thickness. CMT = central macular thickness. FANG = fluorescein angiography.

Author	Time Frame (months)	Study Type and Recruitment	n	C allele frequency	Outcome measures	Results	Other
Brantley et al. 2007[113]	6+	Retrospective, no previous treatment within 6 months	86	0.552326	Snellen VA, baseline FANG	10.5% of CC patients had improvement in VA compared to 53.7% of TT	Caucasian
Lee et al. 2009[114]	9	Retrospective, no previous treatment	156	0.535256	Snellen VA, number of injections	VA was the same between genotypes, but CC had 37% increased risk of requiring additional injections	Caucasian
Imai et al. 2010[121]	6	Prospective	83	NR	BCVA, CRT	Though no CC genotype, TC was more associated with non-response than TT	Japanese
Teper et al. 2010[122]	12	Prospective, 16 patients with no previous treatment	90	0.572222	ETDRS BCVA, CRT	CC patients had reduced improvement in BCVA	Caucasian
Francis et al. 2011[136]	12	Prospective, no previous treatment	64	0.585938	ETDRS VA (letters), number of injections, CMT, CRP	No difference in outcome measures	Caucasian, Lucentis Genotype Study
Kloeckener-Gruissem et al. 2011[123]	12	Prospective, both eyes could be included	243	0.509653	"ETDRS-like" BCVA	Good responders were less likely to be CC than TC or TT	Switzerland
Nischler et al. 2011[124]	11.3	Prospective, no previous treatment	197	0.482234	Snellen distance VA, Radner near VA	More CC patients lost 3 or more lines of VA at distance and near than TT or TC	Austria

Kang et al. 2012[137]	6+	Retrospective, no previous treatment within 6 months	75	0.073333	Snellen VA, CMT, number of injections	No difference in VA or CMT, TC required more injections than TT	Korean
McKibbin et al. 2012[119]	6+	Retrospective, no previous treatment	104	0.495192	ETDR BCVA (letters), OCT	At 6 months, CC patients had a greater improvement in VA than TC or CC	Caucasian
Menghini et al. 2012[125]	24	Retrospective, no previous treatment	204	0.5	logMAR VA (letters)	At 12 and 24 months CT was a predictor of good VA outcome; initial VA response at 3 months was a good predictor of response at 12 months	Switzerland
Orlin et al. 2012[138]	3+	Retrospective, both eyes could be included	143	0.527972	Snellen VA	No difference in genetics between responders and non-responders	USA
Smailhodzic et al. 2012[126]	3	Prospective and retrospective, no previous treatment	420	0.578571	ETDRS VA (letters), age of first injection	VA was significantly better for TT than CC, no difference in age of onset between these groups	White, EUGENDA
Tian et al. 2012[139]	3	Prospective, no previous treatment	144	0.112903	Snellen VA, CRT, max retinal thickness	No difference in treatment outcomes	Chinese
Yamashiro et al. 2012[140]	12+	Retrospective	78	0.166667	BCVA (Landolt C), OCT	No difference in treatment outcomes	Japanese
Abedi et al. 2013[141]	12	Prospective, no previous treatment	211	83% TC+CC	ETDRS VA (letters)	No difference in outcome measures	Caucasian

Chang et al. 2013[142]	6	Retrospective, no previous treatment	102	NR	BCVA, CMT	No difference in treatment outcomes	Korean, 32 patients had polypoidal choroidal vasculopathy
Dikmetas et al. 2013[127]	6+	Prospective, excluded patients who had stable VA with treatment	193	0.582902	ETDRS VA (letters), CMT, FANG lesion width, number of injections	CC was associated with a bad response and TT with a good response	Turkish population
Habibi et al. 2013[143]	6	Prospective, recruited 105 AMD patients but only 70 received treatment	70	0.671429	Snellen VA	No difference in response to treatment; no difference in phenotypes of AMD based on Y402H	Tunisian population
Hagstrom et al. 2013[112]	12	Prospective, No previous treatment	834	0.558153	digital VA (letters), OCT, FANG, number of injections	No difference in outcome measures	CATT, > 98% white
Hautamäki et al. 2013[144]	3 to 6	Prospective and retrospective, No previous treatment	96	0.614583	OCT	No difference in Y402H between responders and non-responders based on fluid seen in OCT	White
Kitchens et al. 2013[145]	9	Retrospective, no previous treatment	97	NR	Snellen VA, OCT	No difference in outcome measures	White
Lotery et al. 2013[111]	12	Prospective, no previous treatment	254	NR	OCT (total retinal thickness)	No difference in treatment outcomes	IVAN, white

Park et al. 2014[146]	5	Prospective, no previous treatment	150	0.10223	ETDRS BCVA (letters), CRT	No difference in treatment outcomes	Korean
Van Asten et al. 2014[147]	3	314 patients retrospective, 77 prospectively; no previous treatment	391	0.571611	ETDRS VA (letters) and Snellen VA	Y402H, lesion type, and lesion size do not predict response vs non-response; predictors included diabetes; non-response at 3 months predicts non-response at 12 months	EUGENDA, Caucasian
Veloso et al. 2014[128]	12	Retrospective, no previous treatment	95	0.526316	Snellen BCVA, CRT	VA improvement occurred in TT and TC, but not in CC at all time points	Brazilian
Matsumiya et al. 2014[148]	3	Prospective, no previous treatment	120	0.133333	BCVA (Landolt C), CRT	No significant difference in VA, Poor resolution of CNV after 3 months with TT and GG genotype in CFH I62V in combination	Japanese
Beykin et al. 2015[115]	48	Retrospective, no previous treatment	45	30% TC+CC	ETDRS BCVA (letters), central subfield and point thickness from OCT, total number of injections	No difference in VA or total number of injections, but CC had thicker central point thickness and central subfield thickness at end of 4 years	Israeli
Piermarocchi et al. 2015[116]	12	Prospective, no previous treatment	94	0.62766	ETDRS BCVA (letters)	CC patients had worse visual outcomes, this was compounded by smoking and hypertension	Caucasian

Kepez et al. 2016[149]	3	Case-control study	109	"rare"	BCVA, CFT, OCT	No difference in treatment outcomes between responders and non-responders	Turkish
Medina et al. 2016[150]	12	Prospective	46	0.48913	BCVA, CRT number of injections	No difference in treatment outcomes	Brazilian
Shah et al. 2016[117]	12	Retrospective, no treatment within 3 months	68	0.544118	Snellen BCVA, CFT	CC had worse visual outcomes and CFT on OCT	USA
Cobos et al. 2017[120]	12	Retrospective, no previous treatment	403	NR for whole population	ETDRS BCVA, CFT, total number of injections	CC had a better initial anatomical response than others; hypertension predicts poor response	Caucasian
Mohamad et al. 2018[151]	6	Prospective, no previous treatment	134	48% TC+CC	Snellen BCVA, CRT	Worse BCVA in CC than TT+CT at 6 months, but no significant difference in change over time	Malaysian
Sengul et al. 2018[118]	60	Retrospective, no treatment within 1 month; excluded non-responders (no change or loss of BCVA after 6 injections) from the study	90*	0.596939	ETDRS BCVA (letters), CMT	Worse BCVA change of CC through all 5 years; Lower number of injections in TT vs CC at 5 years; Lower CMT for TT than TC/CC at 3 and 4 years	Turkish

* Participants reported in genetic analysis were greater than total participants reported

Appendix B. Total DNA Purification Protocol

Prepare/Acquire

- Vortex
- Small centrifuge
- Thermocycler with correct plate – begin a cycle to bring up to temperature

1. Thaw samples of buffy coat at RT in hood
2. Label tubes for buffy coat aliquots (3 total)
3. Once thawed, measure total buffy coat volume using a pipette and divide by 5 to determine how much to include in each aliquot. Remaining buffy coat can be discarded (will save until successful genotyping).
4. Put 2 aliquots in -80'C, and continue protocol with 1 aliquot
5. Spin cells for **5 min** at **2,100 rpm** to pellet
6. Add 200 µL PBS and resuspend
7. Add 20 µL Proteinase K
8. Add 200 µL Buffer AL
9. Mix thoroughly by **vortexing** (15s at level 7) and then incubate in Thermomixer at 56'C for 10 min, 300 rpm
10. Add 200 µL ethanol and mix by **vortexing** (15s at level 7)
11. Pipette sample (~ 700 µL) onto labeled spin column in a collection tube. Centrifuge for **1 min** at **8,000 rpm**. Discard collection tube.
12. Place in new collection tube and add 500 µL Buffer AW1. Centrifuge for **1 min** at **8,000 rpm**. Discard collection tube.
13. Place in new collection tube and add 500 µL Buffer AW2. Centrifuge for ***3 min*** *at* ***13,400 rpm***. Discard collection tube.
    a. Column must be dry - if rewet, then respin for 1 min to prevent residual ethanol
14. Place in new collection tube and add 100 µL Buffer AE to column.
    a. **Incubate** at RT for 1 min.
    b. Centrifuge for **1 min** at **8,000 rpm**. Transfer elutate to final storage container labeled A for first elution.
15. Repeat step 14. Transfer 20 µL to a tube to bring to genomics center and transfer remaining ~80 µL to final storage container labeled B for second elution.
16. Store samples in -80'C.

Appendix C. Impact of Visual Impairment (IVI) Survey Questions

The nine questions that make up the reading and assessing subscale of the IVI survey and the five possible answers patients could select.

In the past month:

1. **How much has your eyesight interfered with your ability to see and enjoy T.V.?**
2. **How much has your eyesight interfered with shopping? (finding what you want and paying for it)**
3. **How much has your eyesight interfered with generally looking after your appearance? (face, hair, clothing etc.)**
4. **How much has your eyesight interfered with opening packaging? (for example, around food, medicines)**
5. **How much has your eyesight interfered with reading labels or instructions on medicines?**
6. **How much has your eyesight interfered with operating household appliances and the telephone?**
7. **How much has your eyesight interfered with reading ordinary size print? (for example newspapers)**
8. **How much has your eyesight interfered with getting information that you need?**
9. **How much has your eyesight interfered with recognizing or meeting people?**

Answers:

1. **Not at all**
2. **A little**
3. **A fair amount**
4. **A lot**
5. **Don't do this for other reasons (Not an option for question 9)**