Macrophage Migration Inhibitory Factor (MIF) Promoter Polymorphisms in Vitreoretinal Disease

Thesis

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Abstract

Proliferative Vitreoretinopathy (PVR) is an inflammatory, fibrotic condition that develops after 5-10% of rhegmatogenous retinal detachments and is the main cause of failure of retinal detachment repair.[1-4] Though there are several known risk factors for PVR, it is not known which patients will develop PVR following RD surgery. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that is an important mediator of inflammation throughout the body.[5-8] MIF polymorphisms, specifically –794 CATT repeat (rs5844572) and –173 G/C promoter (rs755622), lead to an increased expression of MIF and have been linked with disease severity in a multitude of systemic diseases including systemic lupus erythematosus (SLE)[9], rheumatoid arthritis (RA)[10-12], juvenile idiopathic arthritis (JIA)[13], sarcoidosis[14], scleroderma[15], and spinal cord injury, among others. MIF vitreous levels are shown to be elevated in the vitreous and subretinal fluid of patients with PVR.[16,17] Our research aims to determine if the presence of specific MIF promoter polymorphisms leads to an increased risk of PVR development or ERM formation.

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Chapter 1. Introduction

Section A: Proliferative Vitreoretinopathy Background

Proliferative vitreoretinopathy (PVR) is the main cause of failure of retinal detachment surgery. It is estimated to occur following 5-10% of rhegmatogenous retinal detachments.[1] PVR can also occur following intraocular surgery or penetrating ocular trauma. Common risk factors in the presence of a retinal detachment include large retinal breaks or tears, vitreous hemorrhage, multiple previous surgeries, history of trauma, inflammation, long duration of RD, and pre-existing signs of localized PVR such as fixed retinal folds.[18] Systemic disease associated with higher risk of PVR includes Wagner's disease, Stickler Syndrome, Marfan syndrome, and Familial Exudative Vitreoretinopathy (FEVR). PVR is more likely to develop in eyes with prolonged inflammation such as post-operative uveitis, or residual intraocular blood.[18] It is generally accepted that the time course for PVR development is between 4-12 weeks after retinal detachment surgery.[1, 4, 18] The hallmark of this disease is formation of fibrocellular membranes in the retina and fibrosis of the retinal tissue.[19] The contractile nature of these membranes contributes to stiffening of the retina and leads to the formation of large retinal folds. As such, PVR can cause severe damage and vision loss. Surgical intervention, to remove the membranes and repair the detachment, is the only available treatment. Currently there are no adjuvant treatments to reliably prevent or ameliorate PVR. In general, PVR leads to

poor visual outcomes regardless of the success of surgical intervention. Current and future research has begun to focus on determining anti-inflammatory mechanisms for PVR prophylaxis and treatment. Most anti-inflammatory mechanisms are aimed at targeting or inhibiting a specific cytokine or immune mediator in order to prevent abnormal wound healing processes after an RD occurs.

The disease pathology is characterized by components of the wound healing response: inflammation, cellular proliferation, and extracellular matrix remodeling.[19] The wound healing process leads to fibrocellular membrane formation. Vitreoretinal traction and membrane contraction result in marked retinal folds and increased scarring. The membranes are composed of retinal glial cells, retinal pigmented epithelium cells, immune cells, hyalocytes, fibrocytes, and myofibrocytes.[19] RPE cells play a major role in the formation of membranes: during a retinal detachment, the photoreceptor layer detaches from the RPE, causing RPE cells to be released into the subretinal space and vitreous cavity.[20] The wound healing response initiates epithelial-mesenchymal transition (EMT). As part of EMT, the cells become more fibroblast-like as they continue to proliferate and migrate. These cells begin to form clusters on the nearby retinal or vitreal surface, thereby contributing to the formation of cellular membranes.[4, 19, 20] Histological analysis of PVR membranes has demonstrated morphological changes in RPE cells, either to macrophage-like cells or fibrocytes.[20, 21] Blood borne macrophages have also been demonstrated to differentiate into fibroblasts and contribute to membrane formation in PVR.[22] In a rabbit model, injection of activated macrophages into the vitreous resulted in fibrovascular proliferation, thereby suggesting an important role of macrophages in the

ocular inflammatory response.[23] The fibroblastic component of proliferated RPE ultimately plays a role in membrane contraction as well: they migrate along the membrane's surface, adhering to the surface and to neighboring cells which causes the cells to stretch resulting in contractile forces.[20] EMT has been shown to be stimulated by growth factors, cytokines, and cell-to-cell signaling mechanisms. Animal models also demonstrate the importance of the RPE for PVR development. Injection of RPE cells, usually with platelet rich plasma into rabbit vitreous induces PVR-like changes and is a prevalent model for the disease.[24]

The main retinal glial cells involved in the pathogenesis of PVR are Müller cells. In healthy retina, Müller cells play an essential role in maintaining the extracellular environment of the retina and form a large portion of the inter-photoreceptor matrix. In retinal detachment, Müller glia proliferate & migrate out of the retina to contribute to membrane formation and mediate the wound healing response.[25, 26] The major mechanism for this transition is reactive gliosis, which is another key player in the disease process: it is characterized by cell hypertrophy and upregulation of intermediate filaments vimentin and GFAP.[19] When a retinal detachment occurs, the Müller cell migrates into the sub-retinal space contributing to sub-retinal fibrosis. The growth of the cell or its processes beneath the retina inhibits regeneration of the photoreceptor outer segment and therefore has a role in photoreceptor cell death.[25] Similar to animal models for macrophages and RPE cells, injection of Müller glia into rabbit vitreous and upregulation of PDGFα result in an increased induction of PVR.[27] It is theorized that Müller cell interactions with RPE cells influence membrane formation during PVR

pathology.[27] Once the membrane has formed from a combination of RPE, Müller cells, fibrocytes, and myofibrocytes, the contractile forces increase. As the force increases and becomes greater than the adhesive force in the retina, the membrane contracts and pulls on the retinal tissue.[20] This mechanism can cause retinal breaks to re-open, new breaks to form, and result in retinal detachment.

Section B: Epiretinal Membrane Background

The formation of epiretinal membranes can also occur in the absence of PVR and are described as idiopathic or secondary ERMs. They are often associated with vitreoretinal traction or posterior vitreous detachments. However, they have demonstrably fewer RPE cells in their make-up.[28, 29] Fibroblast-like cells are a contributing factor in membrane contraction, but they do not originate from RPE cells. Idiopathic ERMs are not associated with retinal breaks or detachments, thus it is unlikely that cells from the neural retina would play a key role in their formation. Mechanism of idiopathic ERM formation results from breaks in the internal limiting membrane (ILM), most commonly as a result of vitreal traction, causing migration of glial cells and astrocytes onto the retinal surface and thus leading to membrane formation.[30, 31] Surface breaks result in glial cell hypertrophy, and remnants of vitreous on the ILM provide a structural component for membrane formation. As a result of this mechanism, it is reasonable that hyalocytes and glial cells (astrocytes and Müller glia) are the main cellular component in idiopathic ERMs.[30] The etiology of ERMs is most easily discerned by their histological composition: ERMs as a result of PVR contain far more RPE cells, while idiopathic ERMs do not.[21] The main cell component of idiopathic epiretinal

membranes are glial cells, or laminocytes specifically.[32] Overall, ERMs can be classified in three groups: idiopathic, inflammatory (due to neovascular diseases, such as PDR or vascular occlusions), and secondary (due to PVR or retinal tears, previous ocular trauma or surgery, or infectious causes).[32] Clinically, idiopathic epiretinal membranes can lead to metamorphopsia, retinal folds, or cystic edema—but these symptoms are relatively mild compared to those associated with PVR.[29]

Section C: Cytokine Involvement

Another important component in the PVR wound healing response is the interaction of growth factors and cytokines. Many research studies have investigated the roles of specific growth factors and their role in initiating the inflammatory response after a retinal detachment. Cytokines such as IL-1, IL-6, TNFα, TNFβ, and IFN-γ have elevated levels in the vitreous of both RD and PVR patients when compared to cadaveric controls.[33] Of this group IL-1 and IL-6 were the most predominant, and IL-6 had higher levels in PVR vitreous than in RD vitreous. This suggests that PVR is largely an inflammatory process.[33] IFN-γ was also more elevated in PVR vitreous when compared to RD and control vitreous. IFN-γ has been demonstrated to upregulate MHC Class II molecule expression in RPE cells.[33] It also plays a role in activating blood-borne immune cells, which infiltrate the retina during the disease and incorporate into the epiretinal membranes.[33] It is likely that many of these cytokines are produced and secreted by both RPE cells and Müller cells.[33] TNFα has been shown to play an important role in activating RPE proliferation and migration after a retinal detachment occurs. PDGF acts as a mitogen and chemoattractant and also mediates cellular contraction. PDGF is

upregulated in Müller cells and RPE cells, and is thought to play an important role in their interaction and the formation of membranes in PVR models.[27, 34] TGFβ is also elevated in PVR vitreous, and is largely involved in tissue fibrosis.[33] A study completed in 2002 by Hinton *et al* demonstrated that hepatocyte growth factor (HGF) and connective tissue growth factor (CTGF) have higher expression levels in PVR membranes of five patients.[35] HGF has mitogenic, morphogenic, and motogenic activities when it is bound by epithelial cells and upregulates production of TGFβ. The mechanism of CTGF is less well-defined but has increased expression in the presence of TGFβ and is thought to be involved with tissue fibrosis. CTGF expression levels are higher in fibrotic diseases such as lung, renal, and myocardial fibrosis, atherosclerosis, and systemic sclerosis.[35] Vascular endothelial growth factor (VEGF) has also been implicated to interact with PDGF signaling during PVR pathology. VEGF is an important molecule in inflammation and has been demonstrated in a number of other retinal diseases, including proliferative diabetic retinopathy, retinopathy of prematurity, and wet macular degeneration. Anti-VEGF medications are proven to be effective in treatment of some of these diseases and may also be useful in treating macular edema. In 2014, Pennock *et al* investigated the effectiveness of aflibercept, a VEGF trap, in preventing PVR in a rabbit model.[24] Their results demonstrated that aflibercept significantly reduced the PVR score and prevented retinal detachment. In an experimental model of PVR, aflibercept prevented loss of retinal function and also maintained its functional integrity, based on scotopic flash ERG. Histologically, aflibercept prevented formation of ERMs and distortion of the neural retina.[24]

Section D: Cytokine Polymorphisms

Current research has aimed at determining a predictive model for the development of PVR after retinal detachment. A preliminary study by Sanabria Ruiz-Colmenares *et al* analyzed single nucleotide polymorphisms (SNPs) for TNF-α, TGF-β1, IFN-γ, IL-6, and IL-10 among retinal detachment patients, PVR patients, and ethnically similar controls. Their results demonstrated a significance for the TGF-β1 genotype between PVR compared to RD.[36] The Retina 4 Project conducted an extensive research study in Spain aimed at determining genetic markers for prediction of PVR. Their work suggests that genetic profiling in combination with clinical risk factors may produce useful prediction of which patients are predisposed to develop PVR and modify treatment accordingly.[37, 38] Their research examined 196 SNPs in 30 candidate genes, and examined genotyping among RD and RD/PVR patients by testing multiple different predictive models. Two MIF polymorphisms were among the list of SNPs analyzed: rs4820571 and rs738806; however, neither SNP had significant findings.[37] They identified 14 individually significant SNPs; among these, only the TNF locus was the best individual predictor because it was identified by each of the three best predictive models. TNF-α is a known inflammatory mediator with increased synthesis in PVR, and regulates expression of survival factors that protect RPE cells from apoptosis.[39] Further analysis of common tag SNPs within the TNF locus demonstrated 3 significant single associations: two in the lymphotoxin alpha (LTA) gene, and one in the natural killer receptor p30. Only the rs2229094 (within LTA gene) remained significant after false discovery rate analysis, suggesting this is a functional polymorphism for TNF-locus.[39] In a replication phase of

the project, the SMAD7 gene and TNF locus both demonstrated a repeated result of a significant association with PVR.[40] The replication study also included another MIF polymorphism, rs1007888 in their analysis, which had a corrected p value=0.2306 in the discovery stage.[40]

The Retina 4 Project has also suggested an association between BAX and BCL-2 polymorphisms with development of PVR after retinal detachment. Retinal ischemia triggers expression of BCL-2 which is likely involved in photoreceptor loss after retinal detachment. Both BAX and BCL-2 are involved in the tp53 apoptosis pathway; it has been theorized that deregulation of apoptosis leads to abnormal wound healing responses.[41] There was no significant difference in the geographic distribution of the genotypes. Genotype frequency for the BAX polymorphism had an increased risk (OR= 1.8, CI: 1.11-2.95) for PVR in the Spanish population. In the global sample, the BAX heterozygous genotype had an increased risk of PVR (OR=1.72, CI: 1.23-2.51). Results for a BCL-2 promoter polymorphism demonstrated a decreased risk of PVR (OR=0.50, CI: 0.43-0.96) in southern countries.[41] Analysis of a tp53 codon 72 polymorphism demonstrated an increased risk for PVR in Spanish and Portuguese patients. The same result was seen in the Netherlands population but not the United Kingdom subgroup.[42] Also implicated in tp53 pathways is murine double min protein 2 (MDM2), which is an important regulator of tp53 mechanisms. The T309G polymorphism leads to increased MDM2 expression and subsequent decrease in the tp53 pathway.[43] Both the Spain and Portugal populations had a significant association with the polymorphism and increased risk of PVR (OR=5.4), and the UK and Netherlands population had an odds ratio of 7.3;

overall, the odds ratio was 5.9 for the global European sample.[43] Table 1 lists a summary of significant SNPs associated with predictive models of PVR.

Gene	SNP	Details	Population	Results	Ref
EGF	rs11568943	30 candidate genes, identified 196 common tag SNPs under specified parameters	138 PVR patients, 312 RD patients	Adj p=0.2142	Retina 4 Project[37]
FGF2	rs9990554			Adj p=0.0324	
HGF	rs5745687			Adj p=0.0540	
IL1RN	rs1688072			Adj p=0.0436	
IL1RN	rs973635			Adj p=0.0436	
MCP1	rs3760396			Adj p=0.0165	
MMP2	rs1561220			Adj p=0.1936	
NFκBIA	rs17103274			Adj p=0.0234	
PDGFRα	rs7656613			Adj p=0.0905	
PI3KCG	rs6961244			Adj p=0.2676	
SMAD3	rs8032802			Adj p=0.1936	
SMAD7	rs7226855			Adj p=0.0216	
TGFβ2	rs2796821			Adj p=0.0480	
TNFα	rs2857706			Adj p=0.0054	
Replicated **EGF** **FGF2** **HGF** **IL1RN** **IL1RN** **MCP1** **MMP2** **NFκBIA** **PDGFRα** **PI3KCG** **SMAD3** **SMAD7** **TGFβ2** **TNFα**	 rs11568943 rs9990554 rs5745687 rs1688072 rs973635 rs3760396 rs1561220 rs17103274 rs7656613 rs6961244 rs8032802 rs7226855 rs2796821 rs2857706	Validated previous predictive models (Retina 4 Project listed above) and incorporated clinical variables (such as pre-existent PVR, h/o RD, FHx RD, lens status, race)	546 patients	No significant difference observed from original study (above; p=0.298), diagnostic accuracy of 60.98%	Rojas[38]
IL-10	rs17015767				
IL1RN	rs1688072				
MIF	rs4820571 rs738806				
MMP2	rs243840				
NFKB1	rs997476				
NFKBIA	rs17103274 rs3138056				
NFKBIB	rs3136646				
TGFB1	rs2241715				
TGFB2	rs2796821				
TNF	rs2229094				

Table 1: Summary of SNPs in PVR Continued

Table 1 continued

Gene	SNP	Details	Population	Results	Ref
TGFβ1	codon 10, codon 25	selected cytokine gene polymorphisms	27 RD, 31 PVR, 46 control		Sanabria Ruiz-Colmenares[36]
TNF locus	rs2857706 (LTA) rs2229094 (LTA) rs2256974 (NCR3)	same as previous studies[37, 38]	138 PVR patients, 312 RD patients	Only rs2229094 in LTA gene remained significant after false discovery rate analysis	Retina 4 Project[39]
TNFα	rs2857706 rs2229094 rs2256974 rs909253 rs199964 rs1800629	Replication study of TNF locus	546 patient samples (151 cases, 395 controls)	Significant association in SMAD7 and TNF locus genes after stringent statistical analysis	Retina 4 Project[40]
SMAD7	rs6507877 rs7226855				
BCL-2	rs2279115	Same as previous studies	555 total samples (134 PVR): from Spain, Portugal, UK, Netherlands	No difference in genotype frequency across subpopulations; BCL-2 OR=0.95 (UK, Netherlands) OR=0.69(0.43-0.96; global)	Retina 4 Project[41]
BAX	rs4645878			OR=1.66 (UK, Netherlands) OR=1.8 (1.11-2.95; Spain/Portugal) OR=1.72 (1.23-2.51; global)	

continued

Table 1 continued

Gene	SNP	Details	Population	Results	Ref
tp53	rs1042522	Compare genotype distribution in RDPVR patients	270 patients from UK/Netherlands, 271 patients from Spain/Portugal	Significant association in Spain and Portugal populations ($p<0.05$), but not in UK and Netherlands	Retina 4 Project[42]
MDM2	rs2279744	Analyzed genotype frequency of MDM2 and TNF SNPs	Patient population from Spain, Portugal, UK, and Netherlands	Spain and Portugal: OR=5.4 for MDM2 SNP UK and Netherlands: OR=7.3 Global European: OR=5.9	Pastor-Idoate[43]

Section E: Macrophage Migration Inhibitory Factor (MIF)

Macrophage migration inhibitory factor (MIF) has been identified and widely accepted as a regulator of inflammation and may play a significant role in mediating inflammatory disease.[5-7] MIF plays a role in both innate and acquired immunity[8] and has been shown to be expressed by every cell type in the body, with highest levels of expression in the liver, kidney, anterior pituitary gland, brain and various immune cells.[5, 44, 45] It is secreted by both T cells and macrophages, in addition to the anterior pituitary, and is thought to play a major role in regulation of additional inflammatory cytokines.[5, 46] Secretion is induced by LPS, TNF-α and IFN-γ and leads to increased macrophage adherence and phagocytosis.[46] MIF is known to regulate both T-cell and macrophage activation, and is able to override the effect of glucocorticoids on cytokines.[8, 44, 47] Low concentrations of glucocorticoids stimulate MIF release. MIF expression inhibits the

effect of glucocorticoids on cytokine expression, including TNFα, IL-1β, IL-6, and IL-8.[44] In addition, MIF bypasses the function of tp53 tumor-suppressor protein. Cells containing a tp53 mutation are capable of proliferation in the presence of DNA damage, thereby leading to potentially oncogenic mutations. Studies have shown that the presence of MIF results in increased cell survival.[8, 45, 48]

Extensive research has investigated the role of MIF in a myriad of pathology. In particular, two MIF promoter polymorphisms (now known as rs755622 and rs5844572) have been found to have associations with human disease. Donn *et al*[13] identified a single-nucleotide polymorphism (SNP) at –173 position of the MIF gene (rs755622). The G-to-C transition results in creation of activator protein 4 (AP-4) transcription factor, suggesting that the C allele may alter MIF expression. Their group also demonstrated that the G/C polymorphism may be associated with juvenile idiopathic arthritis (JIA).[13] The distribution of the G/C genotype showed significant differences between the disease group and the control group, in addition to comparison between <5 years of disease onset and >5 years of disease onset (with the latter having higher frequency of the G/C and C/C genotypes), implicating that presence of the C allele is associated with greater risk of JIA.[13] Another polymorphism was identified consisting of a tetra nucleotide CATT repeat in the promoter region at position –794 (rs5844572) by Baugh *et al*.[10] Gene variations consist of 5, 6, 7, or 8 repeats with 8 repeats leading to increased expression of MIF. Genotyping was performed in a group of controls and patients with mild to severe rheumatoid arthritis. The CATT-5 allele was significantly reduced in the disease group, as well as the 5,5 genotype which suggests increased MIF levels are associated with

increased disease severity.[10] A study conducted by Radstake *et al*[12] investigated both CATT (rs5844572) and GC (rs755622) polymorphisms in association with RA disease severity and circulating MIF levels. Patients with GC and CC genotype had higher levels of radiologic damage and both the C allele and 7,7 genotype had higher levels of joint damage. They found that the C allele and CATT-7 allele were predictive of joint damage after 6 years of disease, and were associated with higher levels of circulating MIF.[12] MIF has also been documented to have a significant association with systemic lupus erythematosus (SLE). In a case-control study conducted by De la Cruz-Mosso *et al*[9], their group investigated the relationship between MIF polymorphisms and both MIF and TNF-α serum levels in both SLE and control groups, since MIF is known to regulate expression of TNF-α. They found a significant increase in MIF and TNF-α serum levels in the SLE group, as well as a positive correlation between MIF levels and TNF-α levels. In the CATT genotype (rs5844572), there was a significant difference in genotype distribution among the SLE and control groups (OR=1.83 for 6,7; OR=1.5 for 7 allele). In the G/C genotype (rs755622), there was no significant difference between the disease and controls (OR=1.4 for C allele). They demonstrated a positive correlation between the CATT-7 allele and TNF-α serum levels. The C allele also led to a significant increase in TNF-α.[9] Research conducted by Amoli *et al*[14] demonstrates a link between MIF and sarcoidosis. Biopsy of cutaneous granulomas in sarcoidosis have been shown to contain MIF, and serum levels of MIF are elevated in sarcoidosis based on earlier studies. Their research found a significant association with the C allele and erythema nodosum secondary to sarcoidosis, when compared with erythema nodosum due to other or

idiopathic causes. Overall, the G/C distribution for the sarcoidosis group was significantly different than the healthy controls and the idiopathic erythema nodosum group.[14]

Research by Wu *et al*[15] has suggested an association between MIF polymorphisms and disease severity in scleroderma. They evaluated the relationship between G/C (rs755622), CATT (rs5844572), and the haplotypes for limited cutaneous scleroderma (lcSSc), the more severe diffuse cutaneous scleroderma (dcSSc), and healthy controls. In the G/C SNP, the C allele was lower in lcSSc than both dcSSc and controls; whereas the G allele was higher in lcSSc than dcSSc and controls. There was no significant association in the CATT genotype group. The C7 haplotype was significantly lower in lcSSc than dcSSc. There was significance between MIF serum levels and disease severity, though levels appeared to be higher in the C7 group when compared to non-C7 haplotypes.[15]

Outside the realm of autoimmune disease, additional research has investigated the role of MIF in cardiovascular disease. In acute myocardial infarction, MIF plays a role in increasing local inflammation after the event in addition to directly activating local inflammatory cells. Inhibition of MIF during this process reduced cell infiltration and post-MI cardiac rupture.[49] During atherosclerosis, MIF levels are significantly elevated and the cytokine is presumed to play a significant role in endothelial cell adhesions and recruitment of additional cytokines. MIF blockade with anti-MIF antibodies leads to a marked reduction in the inflammatory process, specifically reducing local levels of inflammatory cytokines and reducing macrophage content in the atherosclerotic plaque.[50]

MIF gene polymorphisms have been studied in a variety of cardiovascular disease. Both the CATT Repeat (rs5844572) and G/C SNP (rs755622) are associated with increased severity of carotid artery atherosclerosis (CAA).[51] Both the CATT-7 allele and C allele were associated with increased severity of CAA (p=0.001 and p=0.027, respectively), in addition to the C7 haplotype (p=0.003) demonstrating increased disease severity. After multinomial logistic regression, CATT-7 was associated with moderate (OR=1.94 (1.15-3.27), p=0.012) and severe CAA (OR=2.75 (1.62-4.69), p<0.001). The C allele was associated with severe CAA compared to the GG group (OR=2.03 (1.24-3.33), p=0.005).[51] The G/C promoter (rs755622) SNP was also associated with severity of coronary artery disease (CAD) in a Chinese population.[52] Three SNPs within the MIF gene were evaluated: the G/C promoter (rs755622), in addition to rs1007688 in the translation termination codon and rs2096525 in the first intron. For the latter two, no significant associations were found with CAD compared to controls. The G/C SNP (rs755622) had significantly higher frequency of the CC genotype (p<0.001) and C allele (p<0.001) in the CAD population compared to controls.[52] Multivariate logistic regression adjusted for confounding risk factors demonstrated the CC genotype and C allele as risk factors for CAD: CC genotype OR=2.224 (1.239-3.992), p=0.007, C allele OR=1.473 (1.156-1.876), p=0.002.[52] Additional work has also demonstrated an association between the G/C polymorphism (rs755622) and coronary heart disease (CHD) in a Chinese population.[53] Patients with the CC genotype had increased risk of CHD (OR=2.764, CI: 1.295-5.899, p=0.007).[53]

MIF promoter polymorphisms also have significance in cancer. In a meta-analysis of five different studies of different types of cancer (including leukemia[54, 55], prostate cancer[56, 57], and gastric cancer[58]), patients with the MIF C allele had a significantly higher risk of developing cancer, compared to GG genotype.[59] Further subgroup analysis demonstrated significance for "solid" tumors and C/X genotype (OR=2.67), but no significance for "non-solid" tumors. Overall, heterogeneity of the sample was large in all subgroups but decreased to low levels in only prostate cancer samples.[59]

Furthermore, MIF has also been studied in relation to neurologic disease. In a mouse model of spinal cord injury, intraspinal MIF was elevated during the first two weeks post-injury, during the period of glial cell activation and leukocyte recruitment.[47, 60] Injury-induced MIF elevation exacerbates pathology and impairs neurologic function.[61] In MIF-deficient mice, spontaneous motor function recovery and the number of viable neurons improved, with fewer apoptotic neurons at day 1 and 3 post-injury.[47, 61, 62] In a Turkish population, MIF promoter polymorphisms (rs5844572 and rs755622) were associated with multiple sclerosis.[63] Patients with CATT-6,7 genotype had a lower progression index when compared with 6,6 genotype (p=0.026). The CC genotype was associated with a younger age of disease onset when compared to GG or GC (p=0.012 and p=0.009, respectively), and the 5,6 genotype had a later age of disease onset compared to 6,6 (p=0.044).[63] A later study with a Turkish population did not find any significant association between the GC (rs755622) genotype or allele frequency in multiple sclerosis compared to healthy controls.[64] The GC promoter SNP (rs755622) and CATT repeat (rs5844572) have also been associated with disease outcome in

pneumococcal meningitis.[65] Presence of the C allele or 7 allele were associated with unfavorable outcome (C allele: OR=1.9, p=0.003; 7 allele: OR=1.89, p=0.005), respiratory failure (C allele: OR=1.71, p=0.03), and death (C allele: OR=2.6, p=0.01; 7 allele: OR=2.27, p=0.03). The 7 allele was also associated with CRP (p=0.02), ESR (p=0.02), and CSF leukocyte count (p=0.05). After multivariate logistic regression, the 7 allele significantly increased risk of death (OR=5.12, p=0.04).[65] A summary of MIF polymorphism associations with systemic disease is listed in Table 2.

DISEASE	POPULATION	METHOD OF ANALYSIS	ODDS RATIO (95% CI)	REFERENCE
RHEUMATOID ARTHRITIS	primarily Caucasian (Wichita, KS) n=159 control, n=184 RA patients (79 severe, 105 mild)	CATT genotype (5/5 or 5/x v. x/x)	significant in severe RA v. controls; P<0.02	Baugh[10]
			significant decrease in 5,5 for severe RA v. mild RA (p=0.0252); OR=8.2 (1.03-65.6)	
RHEUMATOID ARTHRITIS	Netherlands; n=277 control, n=273 RA patients	x/7+7,7 v. x/x	no significance, OR=1.00 (0.67-1.48)	Radstake[12]
		7 allele v. x allele	no significance in disease, OR=1.03 (0.72-1.48); higher joint damage in 7 allele (p=0.03)	
		GG v. GC	no significance (p=0.884), OR=1.08 (0.74-1.60)	
		GG v. CC	no significance, OR=1.16 (0.48-2.79)	
		GG v. GC plus CC	higher levels of joint damage in GC,CC group (p<0.001)	
		G v. C allele	no significance in disease, OR=1.09 (0.79-1.49); higher joint damage in C allele (p=0.006)	
		xx v. C7 haplotype	p=0.59, OR=1.30 (0.88-1.93)	

Continued

Table 2: MIF Polymorphism Summary in Systemic Disease

Table 2 continued

DISEASE	POPULATION	METHOD OF ANALYSIS	ODDS RATIO (95% CI)	REFERENCE
RHEUMATOID ARTHRITIS	western Mexican population; n=210 control, n=226 RA	CATT genotype	significant for 6,7 genotype in RA v. control, OR 1.65 (p=0.048)	Llamas-Covarrubias[11]
		CATT allele	7 allele higher in early onset RA v. control, p=0.034; 7 allele assoc. with higher disease activity, p=0.037	
		GC genotype	no significance (data not shown)	
		G v. C allele	C allele assoc. with higher disease activity, p=0.025	
		xx v. C7 haplotype	no significance (data not shown)	
JUVENILE IDIOPATHIC ARTHRITIS	UK population; n=172 controls, n=117 JIA	GC genotype distribution	significant for JIA onset <5years v. control; p=0.04	Donn[13]
			significant for JIA onset >5years v. control; p=0.001	
		G v. C allele	significant for JIA v. control; p=0.0005, OR=2.3 (1.34-3.86)	

continued

Table 2 continued

DISEASE	POPULATION	METHOD OF ANALYSIS	ODDS RATIO (95% CI)	REFERENCE
SYSTEMIC LUPUS ERYTHEMATOSUS	Mexican-Mestizo population; n=200 control, n=186	CATT genotype distribution	significant for 6,7 genotype; p=0.02, OR=1.83 (1.04-3.19)	De la Cruz-Mosso[9]
		CATT allele distribution	significant for 7 allele; p=0.02, OR=1.5 (1.05-2.13)	
		x,x v. x,7+7,7	significant for SLE v. control, p<0.01, OR=1.86 (1.22-2.84)	
		G v. C allele	significant in SLE v. control; p=0.03, OR=1.40 (1.01-1.93)	
		GG v. GC+CC	significant in SLE v. control; p=0.01, OR=1.64 (1.08-2.48)	
SCLERODERMA	Houston, TX; n=254 control, n=486 scleroderma (203 dcSSc, 283 lcSSc)	5/5 v. 5/X	no significance across disease groups v. control(data not shown)	Wu[15]
		5/X v. X/X		
		5/5+5/X v. X/X		
		G v. C allele	higher C frequency in dcSSc v. lcSSc; p=0.010	
			higher C frequency in control v. lcSSc; p=0.011	
		xx v. C7 haplotype	higher in dcSSc compared to lcSSc, p=0.015, OR=1.94 (1.14-3.32)	

continued

Table 2 continued

DISEASE	POPULATION	METHOD OF ANALYSIS	ODDS RATIO (95% CI)	REFERENCE
ERYTHEMA NODOSUM SECONDARY TO SARCOIDOSIS	northwest Spain; n=122 control, n=98 EN patients (28 d/t sarcoidosis, 70 d/t other etiology)	G v. C allele	C allele higher in EN-sarcoid v. EN-other; p=0.004, OR=3.72 (1.75-7.87)	Amoli[14]
			C allele higher in EN-sarcoid v. control; p=0.0016, OR=2.78 (1.45-5.35)	
		GC genotype distribution	significant for EN-sarcoidosis v. control; p=0.002	
		GG v. GC+CC	EN-sarcoid v. control; p=0.007, OR=3.06 (1.32-7.11)	
			EN-sarcoid v. EN-other; p=0.002, OR=4.16 (1.64-10.50)	
CANCER	meta-analysis of 5 studies (including leukemia, gastric, and prostate cancer); n=1728 control, n=1116 cancer	GG v. GC+CC	higher risk of cancer development for any C genotype; p=0.012, OR=1.89 (1.15-3.11)	Vera[59]
			significant association with solid tumors (gastric, prostate); p=0.010, OR=2.67 (1.26-5.65)	
			higher risk in prostate cancer; p<0.0001, OR=3.72 (2.55-5.41)	

continued

Table 2 continued

DISEASE	POPULATION	METHOD OF ANALYSIS	ODDS RATIO (95% CI)	REFERENCE
CARDIOVASCULAR DISEASE	Carotid artery atherosclerosis (CAA) in Taiwan; n=593 patients (167 mild, 212 moderate, 214 severe)	x/x v. x/7 plus 7/7	increased assoc. with severity of CAA, p=0.001	Lan[51]
		x/x v. x/7 plus 7/7 multinomial logistic regression	assoc. with moderate CAA; p=0.012, OR=1.94 (1.15-3.27); assoc. with severe CAA; p<0.001, OR=2.75 (1.62-4.69)	
		GG v. GC+CC	increased assoc. with severity of CAA, p=0.027	
		GG v. GC+CC multinomial logistic regression	assoc. with severe CAA; p=0.005, OR=2.03 (1.24-3.33)	
		xx v. C7 haplotype	assoc. with increased severity of CAA, p=0.003	

continued

Table 2 continued

DISEASE	POPULATION	METHOD OF ANALYSIS	ODDS RATIO (95% CI)	REFERENCE
CARDIOVASCULAR DISEASE	coronary artery disease in northwestern China; n=320 CAD, n=603 control	MIF SNP rs1007888 & rs2096525	no significant difference in genotype or allele distribution between disease and controls	Luo[52]
		GG v. CC	p=0.008, OR=2.089 (1.206-3.619)	
		G v. C allele	p<0.001, OR=1.53 (1.232-1.900)	
		GG v. CC multivariate logistic regression	p=0.007, OR=2.224 (1.239-3.992)	
		G v. C allele multivariate logistic regression	p=0.002, OR=1.473 (1.156-1.876)	
	coronary heart disease in Chinese Han population; n=138 CHD, n=163 controls	GG v. GC	p=0.007, OR=2.764 (1.295-5.899)	Shan[53]

continued

Table 2 continued

DISEASE	POPULATION	METHOD OF ANALYSIS	ODDS RATIO (95% CI)	REFERENCE
MULTIPLE SCLEROSIS	Turkish population; n=120 controls, n=120 MS patients	CATT genotype distribution	no significant association	Akcali[63]
			6,7 had lower progression index compared to 6,6 (p=0.026)	
			5,6 had later age of disease onset compared to 6,6 (p=0.044)	
		GG v. CC	p<0.001, OR=0.141 (0.049-0.405)	
			younger age of onset in CC (p=0.012)	
		GC v. CC	younger age of onset in CC (p=0.009)	
	Turkish population; n=210 controls, n=153 MS patients	GC genotype distribution	no significant association; p=0.227	Cevik[64]
		GG v. GC+CC	no significance; p=0.324, OR=0.79 (0.49-1.26)	
		GG+GC v. CC	no significance; p=0.179, OR=0.29 (0.04-1.27)	
		G v. C allele	no significant association; p=0.157, OR=0.74 (0.48-1.12)	

continued

Table 2 continued

DISEASE	POPULATION	METHOD OF ANALYSIS	ODDS RATIO (95% CI)	REFERENCE
PNEUMOCOCCAL MENINGITIS	Netherlands; n=461 meningitis patients, n=343 controls	xx v. x/7 plus 7/7	7 allele assoc. with unfavorable outcome; p=0.005, OR=1.89 (1.21-2.96); assoc. with death, p=0.03, OR=2.27 (1.07-4.83)	Savva[65]
		xx v. x/7 plus 7/7 logistic regression	7 allele assoc. with death; p=0.04, OR=5.12 (1.11-23.67)	
		GG v. GC+CC	C allele assoc. with unfavorable outcome; p=0.003, OR=1.9 (1.24-2.92); assoc. with respiratory failure, p=0.03, OR=1.71 (1.06-2.74); assoc. with death, p=0.01, OR=2.6 (1.01-3.78)	

In immunohistochemical studies, Matsuda *et al*[66] has demonstrated expression of MIF in the human corneal epithelium and endothelium, the epithelium of the iris and ciliary body, and further demonstrate MIF expression within the human retina. Their work illustrates that MIF is expressed by astrocytes, Muller glia, and RPE cells.[66] A

research study conducted in Japan demonstrated elevated MIF levels in aqueous humor and vitreous humor in patients with uveitis, whereas the control samples had MIF levels below detection.[67] Levels of MIF in the vitreous demonstrated a significant correlation with the activity of inflammation.[67] In a sample of patients with diabetes, MIF aqueous levels were significantly higher in patients with diabetic retinopathy and correlated with the severity of diabetic retinopathy. There was no significance between MIF levels and current HbA1c.[68] Aqueous levels of monocyte chemotactic protein-1 (MCP-1) also demonstrated a significant correlation with severity of diabetic retinopathy. This suggests that MIF and MCP-1 play a significant role in the recruitment and activity of intraocular macrophages in diabetes.[68] In comparison of MIF vitreous levels in proliferative diabetic retinopathy, MIF levels were significantly higher in PDR than control (idiopathic epiretinal membrane or macular hole) eyes and vitreous levels were higher than serum levels of MIF in PDR, but lower in controls. In addition, the MIF levels correlated significantly with the degree of fibrosis in PDR vitreous and were significantly elevated in the cases of neovascular glaucoma.[69]

The same group has also documented increased MIF levels in PVR vitreous. In comparison between PVR vitreous (grade C2 or worse), retinal detachment, and controls (idiopathic epiretinal membrane or macular hole), MIF levels were significantly elevated in the PVR group.[16] Furthermore, MIF levels were significantly higher in PVR Grade D than Grade C. When compared to MIF serum levels, vitreous levels were significantly higher for both PVR and RD samples but lower in controls. This suggests that MIF expression occurs within the eye.[16]

Additional work by Ricker *et al*[17] measured cytokine levels in subretinal fluid of RD and PVR patients. After univariate logistic regression analysis, 18 different cytokines were significantly elevated in PVR compared to RD. In the predictive model, three cytokines (CCL22, IL-3, and MIF) together with the risk factor of preoperative PVR resulted in the best predictive model of postoperative PVR (AUC=0.93, CI: 0.82-1.04).[17]

Section F: Research Goal

The goal of our research herein is to further evaluate a potential correlation between MIF and PVR after retinal detachment. As described above, MIF polymorphisms have been shown to be associated with greater disease severity in autoimmune, fibrotic, and neurodegenerative disease processes. Research has also indicated that MIF levels may play an important role in intraocular inflammation. We aim to evaluate the hypothesis that disease-associated MIF promoter polymorphisms are more prevalent in patients with retinal detachment and RD/PVR, utilizing idiopathic epiretinal membrane and macular hole patients as pseudo-controls in addition to control samples from the Human Genetics Sample Bank. We will evaluate the genotype, allele, and haplotype frequency of the CATT Repeat (rs5844572) and G/C Promoter (rs755622) SNPs and determine whether they are associated with greater risk of PVR or ERM formation.

Chapter 2: Methods

Section A: Patient Recruitment & Sample Collection

Patients were recruited from The Ohio State University Wexner Medical Center in Columbus, Ohio, the Cincinnati Eye Institute in Cincinnati, Ohio, and Mary Lanning Health Care in Hastings, Nebraska. Patients with a history of retinal detachment, with or without PVR, or idiopathic macular hole or epiretinal membrane were consented with permission from their surgeon. Peripheral blood samples were collected prior to surgery and anesthesia. Vitreous humor samples were collected during surgery. Some patients were consented after the date of the original surgery; these patients did not have any vitreous collected, and blood samples were obtained at the office visit. Once informed consent was obtained, a brief medical history was obtained from each patient containing information on demographics, history of systemic disease (such as autoimmune disorders and connective tissue disease), and ocular history. Further exam data was obtained from the surgeons including predisposing factors to RD, history of cataract surgery, degree of myopia, and a PVR diagnosis. Patients were followed at 3- and 6-month exams for evaluation of PVR development. Diagnosis of the subject was made at time of enrollment before the surgery. They were additionally evaluated at the 3- and 6-month visit to determine if PVR had developed post-operatively. The following grading scale, developed by the Retina Society was used(Table 3)[1]:

GRADE	NAME	CLINICAL SIGNS
A	Minimal	Vitreous haze, vitreous pigment clumps
B	Moderate	Wrinkling of inner retinal surface, rolled edge of retinal break, retinal stiffness, vessel tortuosity
C	Marked	Full thickness fixed retinal folds C1: one quadrant C2: two quadrants C3: three quadrants
D	Massive	Fixed retinal folds in four quadrants D1: wide funnel shape D2: narrow funnel shape D3: closed funnel

Table 3: PVR Grading Scale- The Retina Society

Two hundred DNA control samples were obtained from the OSU Human Genetics Sample Bank for analysis. These control samples are part of the "normal control population", mostly from central Ohio. All patient and lab data was entered into REDCap Software for record collection and quality control.

Peripheral blood samples were spun down in a centrifuge within 30 minutes of collection at 2000g for 10-15 minutes with proper biosafety technique. After the centrifuge, plasma was aliquoted out in 1 mL vials and stored in a -80° C freezer.

Processed blood was sent to the OSU Human Genetics Sample Bank for genomic DNA isolation. DNA was extracted using a simple salting out procedure, described by Miller *et al*.[70] Vitreous fluid samples were processed immediately after collection: samples were spun down in a centrifuge at 14000g for 15 minutes within a biosafety cabinet. The sample was aliquoted into 150 uL tubes and stored at -80° C.

Section B: SNP Analysis

PCR was performed with two different protocols for each polymorphism. For the CATT repeats (rs5844572), PCR was run in a 15 uL reaction volume with 20 ng of DNA. Primer sequences were as follows: forward 5'-CTATGTCATGGCTTATCTTC-3'; reverse 5'-TCCACTAATGGTAAACTCGG-3'. The reactions were run at 95° C for 10 min, denatured at 94° C for 30 sec, and cycled through 53° C for 45 sec, and 72° C for 1 min for 44 cycles. Final step was 72° C for 1 min, then a 4° C hold. Samples were analyzed on a 1% agarose gel stained with ethidium bromide, which was run at 100 V for 20-40 minutes. Imaging of the gel with ultraviolet light was used to confirm successful PCR, indicated by bands between 115 to 127bp (Figure 1).

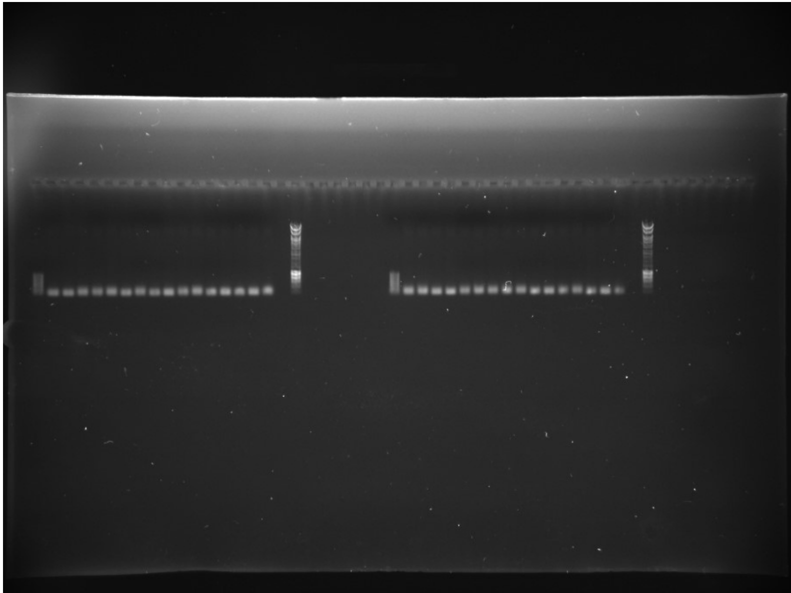

Figure 1: Sample Agarose Gel Image

The samples were sent to the OSU Nucleic Acid Core for capillary electrophoresis using an Applied Biosystems 3730 DNA Sequencer. Genotype of each sample was determined with the following key: 115bp = 5 CATT repeats, 119 bp = 6 CATT repeats, 123 bp = 7 CATT repeats, 127 bp = 8 CATT repeats (Figure 2).

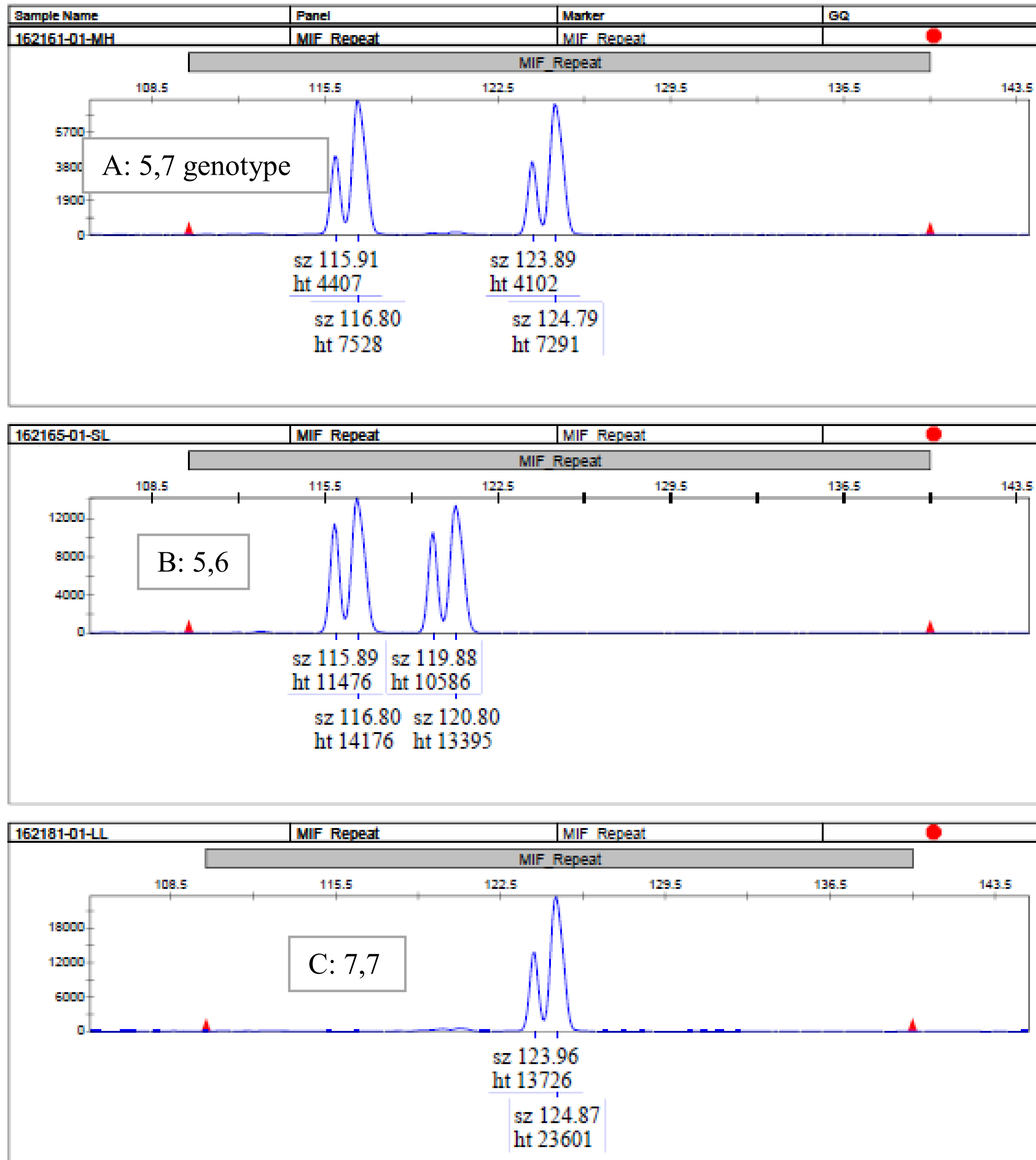

Figure 2: Sample CATT (rs5844572) Sequencing Result

Analysis of the G-C SNP (rs755622) was performed using a Taqman Genotyping protocol. The reactions were 10 uL each with 10 ng of DNA sample. The assay mix used contained forward and reverse primers, and two quenching dyes: VIC-MGB and FAM-

MGB which bind to the C and G alleles, respectively, in the commercially available design. The sequence of the dye is 5'-TGGAGAACAG[G/C]TTGGAGC-3'. Originally, our lab utilized custom-ordered quenching dyes: for this method, the VIC-MGB bound to the G allele and the FAM-MGB bound to the C allele. This method was no longer used when the dyes became commercially available. Primer sequences are as follows: forward 5'- CCGGAACAGGCCGATTTCTAG -3'; reverse 5'- GCAACCGCCGCTAAGC -3'. Real time PCR was performed at 95° C for 10 min, then 40 cycles at 95° C for 15 sec and 60° C for 1 min, and a final hold at 4° C. Allelic discrimination plots were used for sample analysis. Results of the data were analyzed to determine whether the samples were homogenous (VIC or FAM dye) or heterogenous- each indicating a different genotype (Figure 3).

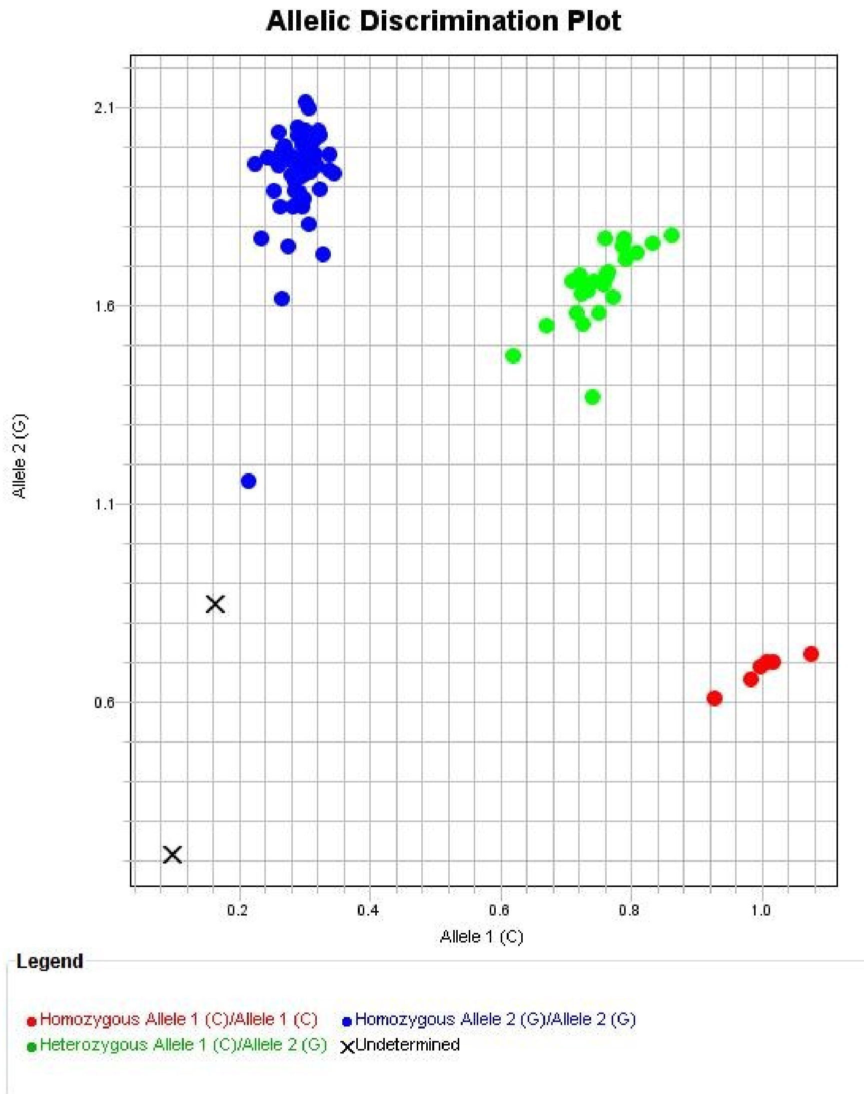

Figure 3: Sample GC Allelic Discrimination Plot

Section C: Data Analysis & Statistics

Samples were grouped based on diagnosis: macular hole (MH), epiretinal membrane (ERM), retinal detachment (RD), and retinal detachment with PVR (RD+PVR), grades B-D in comparison to our control patient samples. Statistical analysis was performed using SPSS Software, using the Pearson Chi square test or Fisher's Exact

Test when expected count of cells was less than five. The odds ratio and 95% confidence interval for PVR and genotype was determined for each diagnosis. Statistical significance was determined based on an alpha value of 0.05.

Chapter 3: Results

Section A: Patient Demographics

Control samples (n=201) were collected from the Human Genetics Sample Bank in Columbus, Ohio. Study participants (n=472), consisting of ERM (n=106), MH (n=47), RD (n=189), and RD-PVR (n=127) diagnoses. Demographics are listed in Table 4.

Study Patients			Controls		
Age	**count**	**percent**	***Age***	**count**	**percent**
18-39	30	6.4%	*18-39*	27	13.4%
40-49	45	9.6%	*40-49*	23	11.4%
50-59	98	20.9%	*50-59*	85	42.3%
60-69	172	36.7%	*60-69*	57	28.4%
70-79	99	21.1%	*70-79*	7	3.5%
80+	25	5.3%	*80+*	2	1.0%
total	469	100.0%	*total*	201	100.0%
Gender	**count**	**percent**	***Gender***	**count**	**percent**
Female	187	39.6%	*Female*	108	53.7%
Male	285	60.4%	*Male*	93	46.3%
total	472	100.0%	*total*	201	100.0%
Race	**count**	**percent**	***Race***	**count**	**percent**
Caucasian	420	89.0%	*Caucasian*	180	89.6%
African American	40	8.5%	*African American*	16	8.0%
Asian	6	1.3%	*Asian*	2	1.0%
Multiple races	1	0.2%	*Hispanic*	1	0.5%
Unknown	5	1.1%	*Middle Eastern*	2	1.0%
total	472	100.0%	*total*	201	100.0%
Smoking Status	**count**	**percent**	**No data collected**		
Never	258	54.7%			
Former	140	29.7%			
Current	70	14.8%			
Unknown	4	0.8%			
total	472	100.0%			

Table 4: Patient Demographics

There were two subjects that de-consented from our study and their records were discarded according to the IRB protocol. Four other subjects were ineligible due to a diagnosis of tractional retinal detachment secondary to proliferative diabetic retinopathy (PDR). A total of 28 patients did not have any blood collected and could therefore not be included in our analysis. Four patients were consented for our study after a previous history of macular hole or epiretinal membrane repair; these patients were also excluded

due to a confounding factor of previous intraocular surgery for fibrotic and tractional conditions. Patients with PVR Grade B-D were included in the Chi square data. All control data were confirmed to comply with the Hardy-Weinberg equilibrium for both the CATT repeat and G/C promoter.

Section B: CATT Repeat (rs5844572) Distribution

Control genotyping was performed with the same method as described above. Genotype and allele frequencies were as follows. 411 patient samples were also analyzed with the CATT genotyping protocol (frequencies listed in Table 5-6).

	control		**RD**		**RD + PVR**		**ERM**		**MH**	
Genotype frequencies	count	percent	count	percent	count	percent	count	percent	count	percent
5,5	16	8.00	13	7.26	6	6.19	4	4.26	2	4.88
5,6	72	36.00	74	41.34	36	37.11	40	42.55	21	51.22
5,7	11	5.50	15	8.38	3	3.09	6	6.38	1	2.44
5,8	0	0.00	1	0.56	0	0.00	0	0.00	0	0.00
6,6	65	32.50	49	27.37	38	39.18	22	23.40	11	26.83
6,7	33	16.50	25	13.97	12	12.37	16	17.02	6	14.63
6,8	1	0.50	0	0.00	1	1.03	0	0.00	0	0.00
7,7	2	1.00	2	1.12	1	1.03	5	5.32	0	0.00
7,8	0	0.00	0	0.00	0	0.00	1	1.06	0	0.00
total	200	100.00	179	100.00	97	100.00	94	100.00	41	100.00

Table 5: CATT Repeat (rs5844572) Genotype Frequencies

	control		RD		RD + PVR		ERM		MH	
allele frequencies (2n)	count	percent	count	percent	count	percent	count	percent	count	percent
5	115	28.75	116	32.40	51	26.29	54	28.72	26	31.71
6	236	59.00	197	55.03	125	64.43	100	53.19	49	59.76
7	48	12.00	44	12.29	17	8.76	33	17.55	7	8.54
8	1	0.25	1	0.28	1	0.52	1	0.53	0	0.00
total	400	100	358	100	194	100	188	100	82	100

Table 6: CATT Repeat (rs5844572) Allele Frequencies

In comparison from *Wu et al*, the control data distribution closely matches that of the normal population and does not deviate from Hardy Weinberg equilibrium (Table 7).

	control		Wu *et al*[15]	
Genotype frequencies	count	percent	count	percent
5,5	16	8.00	9	4.11
5,6	72	36.00	67	30.59
5,7	11	5.50	18	8.22
5,8	0	0.00	0	0.00
6,6	65	32.50	85	38.81
6,7	33	16.50	34	15.53
6,8	1	0.50	3	1.37
7,7	2	1.00	2	0.91
7,8	0	0.00	1	0.46
total	200	100.00	219	100.00

Table 7: CATT Repeat (rs5844572) Control Data v. Wu *et al*

Genotypes were compared by x/x v. 7/7, x/7 v. 7/7 and x/x v. x/7 plus 7/7, where x= 5 or 6. Since the frequency of x/8 genotypes was small, it was combined with the x/7 genotypes for analysis (Table 8).[9, 12] Alleles were analyzed with x v. 7 or 8 format (Table 9).

For the ERM group, x/x v. 7/7 and x/7 v. 7/7 demonstrated a significant difference in genotype distribution when compared to controls (p=0.014 and 0.046, respectively). The OR for x/x v. 7/7 was 6.955 (95% CI: 1.368-35.358), indicating almost a 7-fold increased likelihood of ERM for the 7/7 genotype. For the x/7 v. 7/7 analysis, the OR=6.136 (95% CI: 1.144-32.911). However, there was no significant difference for the x/x v. x/7 plus 7/7 group (p=0.249), possibly indicating that the 7/7 genotype is associated with increased risk of ERM whereas patients that are heterozygous for the 7 allele do not have increased risk of ERM. For the CATT-7,8 allele, the results were borderline insignificant in the ERM group (p=0.058) compared to controls with an OR=1.582 (95% CI: 0.982-2.547). All other analyses had no significant associations.

CATT Repeat Genotype	**x/x v. 7/7**		**x/7 v. 7/7**		**x/x v. x/7 plus 7/7**	
	Chi square (p value)	OR (95% CI)	Chi square (p value)	OR (95% CI)	Chi square (p value)	OR (95% CI)
ERM v. control	**(0.014)***	**6.955 (1.368-35.358)**	**(0.046)***	**6.136 (1.144-32.911)**	1.33 (0.249)	1.381 (0.797-2.393)
MH v. control	(>0.999)*	N/A	(>0.999)*	N/A	0.808 (0.369)	0.670 (0.279-1.610)
RD v. control	(>0.999)*	1.133 (0.157-8.156)	(>0.999)*	1.154 (0.155-8.58)	0.002 (0.963)	0.989 (0.613-1.595)
PVR v. control	(>0.999)*	0.981 (0.088-10.984)	(>0.999)*	1.406 (0.119-16.581)	1.191 (0.275)	0.709 (0.382-1.316)
RD v. PVR	(>0.999)*	1.156 (0.103-12.951)	(>0.999)*	0.821 (0.069-9.700)	1.070 (0.301)	1.393 (0.742-2.617)

Table 8: CATT Repeat (rs 5844572) Genotype Chi Square Results

(Key: *= Fisher's Exact Test)

CATT Repeat Allele	x v. 7,8 allele	
	Chi square (p value)	OR (95% CI)
ERM v. control	*3.592 (0.058)*	*1.582 (0.982-2.547)*
MH v. control	0.914 (0.339)	0.669 (0.291-1.534)
RD v. control	0.000 (0.989)	0.997 (0.644-1.543)
PVR v. control	0.986 (0.321)	0.750 (0.424-1.326)
RD v. PVR	0.929 (0.335)	1.330 (0.744-2.377)

Table 9: CATT Repeat (rs5844572) Allele Chi Square Results

Section C: GC Promoter (rs755622) Distribution

Control genotyping was performed with the same method as described above. Genotype and allele frequencies were as follows. 411 patient samples were also analyzed with the GC genotyping protocol (frequencies listed in Table 10-11).

	control		**RD**		**RD + PVR**		**ERM**		**MH**	
Genotype frequencies	count	percent	count	percent	count	percent	count	percent	count	percent
GG	138	68.66	119	66.11	70	72.92	61	64.89	30	73.17
GC	57	28.36	53	29.44	22	22.92	22	23.40	9	21.95
CC	6	2.99	8	4.44	4	4.17	11	11.70	2	4.88
total	201	100	180	100	96	100	94	100	41	100

Table 10: GC Promoter (rs755622) Genotype Frequencies

	control		RD		RD + PVR		ERM		MH	
allele frequencies (2n)	count	percent	count	percent	count	percent	count	percent	count	percent
G	333	82.84	291	80.83	162	84.38	144	76.60	69	84.15
C	69	17.16	69	19.17	30	15.63	44	23.40	13	15.85
total	402	100	360	100	192	100	188	100	82	100

Table 11: GC Promoter (rs755622) Allele Frequencies

The control population from the work by *Wu et al* closely matches our control population for the GC data as well (Table 12).

	control		Wu *et al*[15]	
Genotype frequencies	count	percent	count	percent
GG	138	68.66	149	65.64
GC	57	28.36	72	31.72
CC	6	2.99	6	2.64
total	201	100	227	100

Table 12: GC Genotype (rs755622) Control Data v. Wu *et al*

Chi square analysis was completed for GG v. CC, GC v. CC, and GG v. GC plus CC (Table 13).[9, 14, 59] Allele chi square results are listed in Table 14. Genotype frequency for the ERM group was significantly higher for the CC genotype in ERM cases than controls when compared to both GG and GC (p=0.004 and 0.004, respectively). The odds ratio for GG v. CC was 4.148 (95% CI: 1.467-11.727), indicating a 4-fold increased risk for ERM development. Alternatively, OR=4.75 (95% CI: 1.566-14.409) for the GC v. CC group, also indicating a higher risk of ERM development. Yet again, in the GG v. GC plus CC group, there was no significant association for the ERM diagnosis (p=0.520).

Allele frequency for the C allele was seemingly elevated for the ERM group, though the results did not reach statistical significance (p=0.073).

GC Promoter Genotype	GG v. CC		GC v. CC		GG v. GC plus CC	
	Chi square (p value)	OR (95% CI)	Chi square (p value)	OR (95% CI)	Chi square (p value)	OR (95% CI)
ERM v. control	**8.173 (0.004)**	**4.148 (1.467-11.727)**	**8.425 (0.004)**	**4.75 (1.566-14.409)**	0.413 (0.520)	1.185 (0.706-1.989)
MH v. control	(0.638)*	1.533 (0.295-7.970)	(0.339)*	2.111 (0.368-12.123)	0.327 (0.568)	0.803 (0.378-1.704)
RD v. control	0.651 (0.420)	1.559 (0.526-4.622)	0.488 (0.485)	1.49 (0.484-4.585)	0.171 (0.680)	1.095 (0.711-1.687)
PVR v. control	(0.734)*	1.353 (0.369-1.955)	(0.470)*	1.727 (0.444-6.713)	0.413 (0.521)	0.838 (0.487-1.439)
RD v. PVR	(>0.999)*	1.153 (0.335-3.970)	(>0.999)*	0.863 (0.235-3.166)	0.918 (0.338)	1.308 (0.755-2.265)

Table 13: GC Genotype (rs755622) Chi Square Results (Key: *= Fisher's Exact Test)

GC Promoter Allele	G v. C allele	
	Chi square (p value)	OR (95% CI)
ERM v. control	*3.221 (0.073)*	*1.475 (0.963-2.257)*
MH v. control	0.083 (0.773)	0.909 (0.476-1.736)
RD v. control	0.396 (0.529)	1.127 (0.777-1.633)
PVR v. control	0.134 (0.715)	0.916 (0.574-1.464)
RD v. PVR	0.737 (0.391)	1.230 (0.767-1.972)

Table 14: GC Allele (rs755622) Chi Square Results

Section D: Haplotype Analysis

Haplotype frequency was analyzed across our patient population, evaluating C7 plus C8 v. other haplotypes (Table 15). Results of the Chi square demonstrated a significantly higher risk of ERM for the C7,C8 haplotype (p=0.002, OR=1.901, 95% CI: 1.254-2.883).

Haplotype	XX v. C7, C8	
	Chi square (p value)	OR (95% CI)

ERM v. control	**9.387 (0.002)**	**1.901 (1.254-2.883)**
MH v. control	0.793 (0.373)	0.708 (0.331-1.518)
RD v. control	0.072 (0.788)	1.056 (0.709-1.574)
PVR v. control	0.342 (0.559)	0.859 (0.515-1.432)
RD v. PVR	0.617 (0.432)	1.230 (0.733-2.065)

Table 15: Haplotype Chi Square Results

Section E: Subgroup Analysis

Subgroup analysis was also completed according to grade of PVR. Grades C and D were combined for this analysis due to the lower frequency in each group (frequencies listed in Table 16-19).

Genotype frequencies	**Grade A**		**Grade B**		**Grade C/D**	
	count	%	count	%	count	%
5,5	0	0.00	4	12.12	2	3.17
5,6	9	39.13	10	30.30	25	39.68
5,7	1	4.35	0	0.00	3	4.76
5,8	0	0.00	0	0.00	0	0.00
6,6	12	52.17	14	42.42	24	38.10
6,7	1	4.35	5	15.15	7	11.11
6,8	0	0.00	0	0.00	1	1.59
7,7	0	0.00	0	0.00	1	1.59
7,8	0	0.00	0	0.00	0	0.00
total	23	100.00	33	100.00	63	100.00

Table 16: CATT Genotype (rs5844572) PVR Grade Distribution

Allele frequencies (2n)	Grade A		Grade B		Grade C/D	
	count	%	count	%	count	%
5	10	21.73913	18	27.27273	32	25.39683
6	34	73.91304	43	65.15152	81	64.28571
7	2	4.347826	5	7.575758	12	9.52381
8	0	0	0	0	1	0.793651
total	46	100	66	100	126	100

Table 17: CATT Repeat (rs5844572) Allele PVR Grade Distribution

Genotype frequencies	Grade A		Grade B		Grade C/D	
	count	%	count	%	count	%
GG	16	72.73	26	78.79	43	69.35484
GC	6	27.27	7	21.21	15	24.19355
CC	0	0.00	0	0.00	4	6.451613
total	22	100.00	33	100.00	62	100.00

Table 18: GC Promoter (rs755622) Genotype PVR Grade Distribution

Allele frequencies (2n)	Grade A		Grade B		Grade C/D	
	count	%	count	%	count	%
G	38	86.36	59	89.39	101	81.45
C	6	13.64	7	10.61	23	18.55
total	44	100	66	100	124	100

Table 19: GC Promoter (rs755622) Allele PVR Grade Distribution

Analysis of genotype and allele frequency among PVR Grade demonstrated no significant association, as shown below (Table 20).

PVR Grade	Chi square (p value)
CATT Genotype	11.413 (0.444)*
CATT Allele	2.937 (0.884)*
GC Genotype	2.869 (0.594)*
GC Allele	2.368 (0.302)

Table 20: PVR Grade Chi Square Results

In addition to the above analysis, there was a small cohort of patients initially consented in the study with a retinal detachment that were also diagnosed with ERM or MH (Table 21).

subgroup	*count*
RD/ERM	34
RD/MH	4
RD/ERM/MH	3
PVR/ERM	23
PVR/MH	1
ERM/MH	6
total	71

Table 21: Diagnosis Subgroup Count

Genotype frequencies for the RD/ERM and PVR/ERM groups are below (Table 22-23).

	RD		**RD/ERM**		**PVR**		**PVR/ERM**	
	count	percent	count	percent	count	percent	count	percent
5,5	12	8.63%	0	0.00%	5	6.49%	0	0.00%
5,6	59	42.45%	15	40.54%	29	37.66%	6	33.33%
5,7	11	7.91%	3	8.11%	2	2.60%	1	5.56%
5,8	1	0.72%	0	0.00%	0	0.00%	0	0.00%
6,6	37	26.62%	12	32.43%	32	41.56%	6	33.33%
6,7	18	12.95%	6	16.22%	7	9.09%	5	27.78%
6,8	0	0.00%	0	0.00%	1	1.30%	0	0.00%
7,7	1	0.72%	1	2.70%	1	1.30%	0	0.00%
7,8	0	0.00%	0	0.00%	0	0.00%	0	0.00%
total	139	100.00%	37	100.00%	77	100.00%	18	100.00%

Table 22: RD/ERM and PVR/ERM CATT Repeat (rs5844572) Distribution

	RD		**RD/ERM**		**PVR**		**PVR/ERM**	
	count	percent	count	percent	count	percent	count	percent
GG	95	67.86%	23	62.16%	56	73.68%	12	66.67%
GC	41	29.29%	10	27.03%	16	21.05%	6	33.33%
CC	4	2.86%	4	10.81%	4	5.26%	0	0.00%
total	140	100.00%	37	100.00%	76	100.00%	18	100.00%

Table 23: RD/ERM and PVR/ERM GC (rs755622) Distribution

Statistical analysis was evaluated for RD v. RD/ERM groups and PVR v. PVR/ERM (Grade B-D) for the CATT genotype (rs5844572), CATT allele, GC genotype (rs755622), and GC allele. The results are presented in Table 24.

	CATT Genotype	**CATT Allele**	**GC Genotype**	**GC Allele**
RD v. RD/ERM	5.951 (0.414)*	3.417 (0.348)*	3.901 (0.139)*	1.776 (0.242)
PVR v. PVR/ERM	6.283 (0.363)*	3.845 (0.300)*	1.518 (0.461)*	0.017 (>0.999)

Table 24: ERM Subgroup Results

Chapter 4: Discussion

In this project we aim to determine the presence of an association between certain MIF polymorphisms and idiopathic epiretinal membranes or the development of proliferative vitreoretinopathy after rhegmatogenous retinal detachment. MIF is a well-known mediator of the local inflammatory response, playing a large role in fibrosis, glial cell remodeling, and the wound healing response. It has been suggested to have an important role in various systemic diseases, such as systemic lupus erythematosus[9], rheumatoid arthritis[10-12], sarcoidosis[14], juvenile idiopathic arthritis[13], scleroderma[15], spinal cord injury[47], cardiovascular disease[49, 50], and cancer development[59]. Presence of the 7 or 8 CATT repeat allele leads to higher expression of MIF (rs5844572), and therefore may be a causative factor in disease severity. In the G/C promoter SNP (rs755622), presence of the C allele leads to increased MIF expression and has also be linked with increased disease severity. Furthermore, MIF levels are elevated in aqueous and vitreous of patients with intraocular inflammatory conditions, including uveitis[67], proliferative diabetic retinopathy[69], and proliferative vitreoretinopathy.[16] Because the pathology of PVR is related to the wound healing response and increased fibrosis within the eye, it is reasonable to assume that MIF may play an important role in the development of this disease.

Our data analyzed samples from 201 control patients and a total of 411 study samples. Genotype frequency of the CATT Repeat (rs5844572) were analyzed with an x/x v. 7/7, x/7 v. 7/7, and x/x v. x/7 plus 7/7 model.[9, 12] Allele frequency used the x v. 7 allele model. Since the frequency of the 8 allele and genotype were so low, they were combined with the 7 allele groups for data comparison. For the G/C Promoter (rs755622), we used a GG v. CC, GC v. CC, and GG v. GC plus CC model.[12, 14, 59] Allele frequency was analyzed in a G v. C method. There were eight possible haplotype combinations for our data: G5, G6, G7, G8, C5, C6, C7, and C8. Analysis of the haplotypes utilized XX v. C7, C8 (since the frequency of the C8 haplotype was also significantly lower than other groups).

Our results did not demonstrate significant associations for the MH, RD, and PVR groups. Analysis of the ERM group versus control had significantly higher frequency of the 7/7 genotype when compared to x/x (p= 0.007, OR= 6.955) and x/7 (p= 0.02, OR= 6.136). However, when these groups were combined in the x/x v. x/7 plus 7/7 group, the results were not significant against controls (p= 0.247). In the allele x v. 7,8 analysis for ERM, results were just outside significance (p= 0.058, OR= 1.582). Taken together, this would suggest that presence of the 7/7 genotype results in an increased risk of ERM, though heterozygous genotypes for the 7 allele do not have an increased risk. There was also significantly higher CC genotypes in ERM when compared to GG (p= 0.004, OR= 4.148) and GC (p= 0.004, OR= 4.75) versus the control group, though the GG v. GC plus CC association was not significant (p= 0.52). Similarly, the C allele was close to significance (p= 0.073, OR= 1.475). These results would also indicate that presence of

the CC genotype results in increased risk of ERM, while the GC genotype does not. Evaluation of haplotype frequency demonstrated a significantly higher number of the C7 or C8 haplotype within the ERM group compared to controls ($p= 0.002$, $OR= 1.901$). This finding further affirms that both the C allele and 7 allele, which are known to increase MIF expression levels, are important for disease development.

The results of this work may be limited due to our sample size. Our original goal was to consent 100 patients with PVR, in order to achieve 80% statistical power. Our main analysis had n=97 PVR patients (grade B-D). However, due to the rare frequency of the risk alleles (C allele and 7 or 8 allele), an increase in sample size would help to elucidate the relationship further. Studies such as the Retina 4 Project have looked at a population of over 500 patients, comprised of around 140 PVR cases.[37, 39-42] Increased population size will increase the reliability of predicting PVR susceptibility. Other published work similar to our study model have analyzed haplotypes based on an reconstructed inference model[11, 12, 15], such as the E-M algorithm, using haplotype software like HPlus or Haploview. Future data analysis should incorporate this model in order to increase the accuracy of our data.

Further research within our IRB protocol aims to determine MIF levels within the vitreous of our patients. These levels will be age-matched to the control population, in an attempt to determine a link between disease state and MIF levels. We will also evaluate a correlation between MIF plasma levels and MIF vitreous levels, in order to determine the potential use of MIF as a biomarker in PVR. MIF levels will be compared with the MIF genotype, in order to investigate the association between genotype and MIF expression.

The results of the work discussed here suggest an interesting relationship between MIF polymorphisms and the presence of epiretinal membranes. Current knowledge of idiopathic ERM formation is presumed to be caused by a break in the internal limiting membrane, resulting in proliferation of glial cells on the retinal surface.[29, 30, 32] Because MIF is known to play an important role in tissue fibrosis and inflammatory regulation, it may have a significant role in glial cell hypertrophy during ERM formation. Further research into the relationship of MIF polymorphisms and ERM may include information on the clinical severity. Research completed by other groups has demonstrated an association between MIF polymorphisms and clinical disease factors, such as: length of disease onset in JIA[13], levels of joint damage in RA[12], or correlation of MIF levels with RF and CRP in RA patients.[11] Clinical values for ERM may include best-corrected visual acuity, presence of metamorphopsia, chronicity of the condition, indication for surgery, or post-operative visual acuity. Additional research may help to determine the cause of ERM formation and potential treatment or prevention of significant vision loss.

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