Genetic Epidemiology of Age-Related Macular Degeneration (AMD): The Role of the Complement Component 2 (C2) and Complement Factor B (CFB) Genes in Determining AMD Subphenotypes

PhD Thesis

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary institution.

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Abstract

Age-related macular degeneration (AMD) is the most common cause of irreversible blindness in people over the age of 50 in the developed world. Inflammation has a central role in the pathobiology of AMD; complement pathway dysfunction is thought to induce significant damage to macular cells, leading to atrophy, degeneration and the elaboration of choroidal neovascular membranes. The complement component 2 (*C2*) and complement factor B (*CFB*) genes are among several loci implicated though basic biology and genetic association studies in playing a significant role in determining susceptibility to AMD. The precise manner by which these genes affect AMD risk is unclear and largely theoretical. The purpose of this study was to examine the potential role of *C2/CFB* polymorphic genetic loci in determining the clinical severity of AMD and the manifestation of different AMD subphenotypes. Specifically, it was hypothesised that the *C2/CFB* genes are associated with AMD severity, independent of known AMD risk factors such as the complement factor H (CFH) Y402H polymorphism, smoking, and other plausible covariates.

This research forms part of, and contributed greatly to, the WA Macular Degeneration Study (WAMDS); a cross-sectional clinic-based case series of 1013 AMD cases comprising 675 choroidal neovascularisation (CNV) patients, 71 geographic atrophy patients, and 267 "early" (Age-Related Eye Disease Study [AREDS] grades 1-3) patients. Case-control analyses of subphenotypes utilised either or both of the geographic atrophy and early AMD subsets as 'controls'. All participants were existing patients of the Lions Eye Institute Elsie Gadd Eye Clinic in Nedlands, Western Australia.

DNA samples were genotyped for C2/CFB using a haplotype-tagging set of 19 single nucleotide polymorphisms (SNPs) to capture 78% of the common variation in these genes. Additionally, the DNA samples were also genotyped for the CFH Y402H variant. AMD severity was graded according to the AREDS scale by ophthalmologists upon review of fundus photographs. Smoking and other relevant environmental/medical histories were collected via a comprehensive questionnaire. The multivariate associations of tagging SNPs and AMD phenotypes were tested.

Significant associations between *C2/CFB* genetic variants and AMD subphenotypes were observed. The rs7746553 (P = 0.04), rs3020644 (P = 0.008) and rs4151657 (P = 0.02) variants were associated with neovascular AMD; rs7746553 was also associated with legal blindness (P = 0.04). The rs2072633 (P = 0.002), rs1048709 (P = 0.005) and rs537160 (P = 0.002) variants were associated with neovascular lesion composition. Models were adjusted for appropriate covariates and *CFH* Y402H, and all P values were adjusted for multiple testing.

These results support the hypothesis, suggesting that *C2/CFB* genetic variants are associated with altered/compromised functioning of the alternative complement pathway and with a concomitant increased risk of developing more severe AMD independent of the CFH Y402H variant. Additionally, in establishing this study, I and my collaborators have created a comprehensive and useful resource for current and future AMD research.

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Abbreviations

AIC	Akaike Information Criterion
AMD	Age-related Macular Degeneration
AREDS	Age-Related Eye Disease Study
Arg	Arginine
Asp	Aspartate
BMI	Body Mass Index
bp	base pair/s
C2	Complement Component 2 protein (gene if italicised)
C3a	Complement component 3a
C3b	Complement component 3b
C5a	Complement component 5a
CEU	Caucasian-European
CFB	Complement Factor B protein (gene if italicised), also known as BF or Factor B
CFH	Complement Factor H protein (gene if italicised)
CFP	Colour Fundus Photography
CI	Confidence Interval

CNV	Choroidal Neovascularisation
CRP	Complement Reactive Protein
DA	Disc Areas
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
FDR	False Discovery Rate
GA	Geographic Atrophy
Glu	Glutamate
Gln	Glutamine
His	Histidine
HWE	Hardy-Weinberg Equilibrium
JLIN	Java-based Linkage disequilibrium plotter
kb	kilobase/s
LD	Linkage Disequilibrium (r^2)
LEI	Lions Eye Institute
Leu	Leucine
logMAR	Logarithm of the Minimum Angle of Resolution

LR	Likelihood Ratio
MAF	Minor Allele Frequency
Met	Methionine
MHC	Major Histocompatibility Complex
mRNA	messenger Ribonucleic Acid
n	sample size
NCBI	National Center for Biotechnology Information
NSAID	Non-Steroidal Anti-Inflammatory Drug
OR	Odds Ratio
P	*P* value
PCR	Polymerase chain reaction
r^2	see entry for "LD"
RPE	Retinal Pigment Epithelium
SCR	Short Consensus Repeats
SE	Standard Error
SNP	Single Nucleotide Polymorphism
UTR	Untranslated Region
VA	Visual Acuity

VEGF	Vascular Endothelial Growth Factor
WAMDS	Western Australian Macular Degeneration Study

Glossary

Allele: One of two or more alternative forms of a gene located at the corresponding site of homologous chromosomes

Association: The statistical association of an allele with a phenotypic trait. Statistical dependence between two or more events, characteristics, or other variables.

Bias: Deviation of results or inferences from the truth, or processes leading to such deviation.

Bonferroni correction: A multiple test correction method. The false-positive rate is divided by the number of tests, and this modified number is used to declare any single change to be significant.

Candidate Gene: A sequenced gene of previously unknown function that, because of its chromosomal position or some other property, becomes a candidate for a particular function such as disease determination.

Chromosome: A linear end-to-end arrangement of genes and other DNA, sometimes with associated protein and RNA.

Complex Genetic Disorder: A disease that involves several genetic and environmental factors, and which does not exhibit a classic Mendelian pattern of inheritance.

Confounding variable (confounder): An additional variable that may be responsible for an apparent association between a genotype and a phenotype.

Covariate: A potentially confounding variable controlled for in analysis of

covariance.

Exon: The region of a gene that is present in the final mRNA transcript.

Genome: The total complement of DNA carried by an individual.

Haplotype: A group of nearby alleles that are inherited together.

Hardy-Weinberg equilibrium: The stable frequency distribution of genotypes A/A, A/a, and a/a, in the proportions p^2, 2pq, and q^2, respectively (where p and q are the frequencies of the alleles A and a), that is a consequence of random mating in the absence of mutation, migration, natural selection, or drift.

Heritability: The proportion of population variance in a trait attributable to segregation of a gene or genes.

Heterozygous: Having two different alleles at a specific autosomal (or X chromosome in a female) gene locus.

Homozygous: Having two identical alleles at a specific autosomal (or X chromosome in a female) gene locus.

Intron: A non-coding sequence of DNA that is initially copied into RNA but is spliced out of the final RNA transcript.

Linkage: The tendency for genes that are located close to each other on the same chromosome to be inherited together.

Linkage disequilibrium: Two alleles at different loci that occur together within an

individual more often than would be predicted by random chance. Also called population allelic association.

Locus: Any genomic site, whether functional or not, that can be mapped through formal genetic analysis.

Marker: A segment of DNA with an identifiable physical location on a chromosome and whose inheritance can be followed.

Meta-analysis: A method for combining the results from several independent studies of the same outcome so that an overall *P* value may be determined.

Molecular genetics: The study of the molecular processes underlying gene structure and function.

Monogenic: Controlled by or associated with a single gene.

Oligonucleotide: Short sequence of single-stranded DNA or RNA.

***P* value:** The probability of observing a result as extreme as or more extreme than the one actually observed from chance alone (i.e. if the null hypothesis is true).

Pathophysiology: Derangement of function seen in disease.

Phenotype: The clinical presentation or expression of a specific gene or genes, environmental factors, or both.

Polygenic: Pertaining to the combined action of alleles of more than one gene.

Polymerase chain reaction (PCR): A method for amplifying specific DNA segments that exploits certain features of DNA replication.

Polymorphism: A common variation (>1%) in the sequence of DNA among individuals.

Population stratification: Occurs when cases and controls have different allele frequencies attributable to diversity in background population, unrelated to outcome status. A potential confounding factor in genetic association studies.

Power: The ability of a study to detect an actual effect or difference.

Primer: An oligonucleotide sequence used in a polymerase chain reaction.

Probe: Defines a nucleic acid segment that can be used to identify specific DNA molecules bearing the complementary sequence.

Promoter: The part of a gene that contains the information to turn the gene on or off. The process of transcription is initiated at the promoter.

Recombination: The exchange of genetic material between homologous chromosomes during meiosis, producing a combination of alleles at two distinct loci.

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