

**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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This thesis is presented for the degree of Doctor of Philosophy of
Murdoch University, 2009



Murdoch
UNIVERSITY



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.

Brooke Allison Cattell Longville

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 through 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
<i>P</i>	<i>P</i> 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.

Acknowledgements

This work would not have been possible without the support and wisdom of many people.

First and foremost, I thank my supervisors for providing me with this opportunity. All of you inspired me and guided me through the trials and tribulations of scientific research in a field that was initially rather alien to me.

Professor Lyle Palmer is a visionary who truly embodies all things which make a great scientist; passion, inspiration, motivation and dedication; with a contagious sense of humour as a bonus. Your personality and enthusiasm were incredibly inspiring and although keeping up with you was sometimes a challenge, it was a challenge I relished and needed. I feel we have a quiet understanding and acceptance of each other, and I appreciated being able to develop in the company of a kindred spirit.

The nicest and most approachable academic on the planet would have to be Dr Wayne Greene, who I couldn't bear to part with after completing my honours under his supervision. I thank you for always believing in me and having the uncanny talent of knowing *exactly* when I needed to be saved (and then saving me, or helping me to save myself!). You have often been a last beacon of hope and courage when I felt all was lost. You taught me how to be a practical optimist, and that success can be defined in many ways; happiness is the ultimate success. I hope I can continue to benefit from your mentorship in future, and that my continued intellectual growth is a fitting testament to your excellent tutelage.

I also thank Professor Ian Constable, then-Director of the Lions Eye Institute, for his patience, support and dedication to the cause. I am amazed and humbled that you managed

to squeeze time for me in between your lengthy hours of consultation, surgery and the considerable demands that go hand in hand with being a famous, successful and all-round world class ophthalmologist/researcher. You are superhuman, yet human; always putting your patients first and rarely stopping to rest.

My supervisors' wives also deserve special thanks for tolerating the extra stress I created for their husbands! In particular, Dr Sutapa Mukherjee; for providing special aid and support at critical times, and Dr Elizabeth Constable; for unselfishly allowing her husband to share his knowledge and skills with the world, regardless of the hours involved.

I thank the numerous people working at the Lions Eye Institute; the marvellous ophthalmic photographers Frank Shilton and Chris Barry who kept me sane during many clinic shifts, the many helpful nurses and receptionists (a big shout-out to Daphne especially), the brilliant and accommodating clinic manager Julie Robson, and ophthalmologists Professor Ian McAllister and Dr Tim Isaacs. All of these people went above and beyond their usual responsibilities in assisting me with the study whenever possible. Special thanks go to the personal assistants of Professor Pirooska Elizabeth Rakoczy, Stacey Scaffardi and Natalie Mitchell, and Professor Ian Constable's wonderful assistant Esther McCloskey.

Thanks are due to many people at PathWest, in particular A/A Professor John Beilby and research assistants Michelle Jennens and Gillian Arscott. Extended thanks go out to WA DNA Bank manager Dr Marion Macnish and staff Simone Dowd and Laura Greenwood.

I am indebted to all of my colleagues at the Centre for Genetic Epidemiology and Biostatistics; it was a pleasure to work alongside such intelligent, dynamic and outstanding people. In particular, my wonderful statistical consultants, Dr Pamela McCaskie and Nicole

Warrington, were critical to the success of this project. I thank Nicole for being incredibly patient with me, and dedicating so much time and effort to my analytical plight. Pam, I thank you for being a role model and trusted confidante, your wisdom and empathy got me through many tough times. I thank my mentors; Dr Brenda Powell, Dr Anne Pratt and Dr Marion Macnish; for being there for me at every turn, armed with sound advice, inspiration, and a shoulder to lean on. Special thanks to Marion for going above and beyond in proofreading my thesis. Great thanks are due to the creative and efficient IT team; in particular Chris Williams, Chris Ellis, Kim Carter, CK Leong, Paul White and Declan Lynch; your extraordinary efforts made this study possible, and your professional, friendly and approachable dispositions made it a pleasure to work with you. I am indebted to the many efficient personal assistants of Prof Lyle Palmer; Jeanette Dungey, Ida Handy, Janet Gitsham, Lisa Bayley, and Brenda Loney, who recently received her baptism (largely by way of printing and binding a copy of my thesis). Finally, I thank Matt Cooper for helping me retain my sanity in the late stages of this thesis.

I respectfully acknowledge and greatly appreciate the essential financial and infrastructural support that made both this research and my PhD studentship possible; I thank the Wind Over Water Foundation, Professor Ian Constable and the Lions Eye Institute, A/A Professor John Beilby and PathWest, the UWA Centre for Genetic Epidemiology and Biostatistics, WAIMR and the Australian Government. I gratefully acknowledge the assistance of the Western Australian Genetic Epidemiology Resource and the Western Australian DNA Bank (both National Health and Medical Research Council of Australia National Enabling Facilities). Furthermore, I thank Murdoch University for providing me with the best possible environment and academic support throughout my nine years of

tertiary education. In particular, Associate Professor Robert (Bob) Mead deserves special mention for his critical guidance throughout my undergraduate years, and Dr Wayne Greene, my champion and trusted mentor throughout my postgraduate studies. Outstanding people like Bob and Wayne, combined with a unique, inclusive and intelligent ethos, make Murdoch a truly great university. Murdoch, I thank you for choosing me.

I salute the entire AMD study team; Caroline Adams, Jude Willis, Dr Alex Tan, Dr Xia Ni Wu, soon-to-be-Dr Jing Xiao and Dr Xuefeng Feng. Without your contributions of time and effort this study would not have existed, let alone produced valuable data. Furthermore, your friendship and cooperation saved my sanity countless times; so many things I want to say to you all. To all of my “surrogate grandparents”; the generous participants of the WA Macular Degeneration Study, I thank you for your enthusiasm and patience; it was a joy meeting all of you and being able to share the wealth of knowledge you volunteered. I hope my research may one day benefit your children and grandchildren, and justify our collective sacrifices.

To my loyal and patient friends, I am eternally grateful for your friendship and support. While there are too many wonderful people to mention them all, a select few were particularly influential during critical stages of my PhD. The cheery and intelligent Dr Jacqueline McGlade made me look forward to each day as an undergraduate, and she has been a role model and shining beacon at the end of the PhD tunnel. Soon-to-be-Dr Yazid Abdad was the ultimate KFC lunch buddy, and guaranteed to laugh at even my weakest jokes. Soon-to-be-Dr Roheeth Delima has been an insightful and inspirational influence on me, and I value our numerous lengthy philosophical conversations. And finally, I thank Clinton Hall for giving me a refreshing perspective and moral support; you gave me my

proper second wind when I had been flailing around in a mini-tornado of sorts for too long. I hope I'm worthy and deserving of your continued friendship as, one by one, we head out into the world as real scientists.

Finally, I owe a great deal to my family for helping me weather the storm and survive the hard yards. To my parents Don and Liz; I thank you for giving me the space I needed, and not trying to stop me! To my awesome sister and flatmate Shannon; I cannot count the number of times you have inspired, supported and humoured me. I'm glad we don't have any other siblings, because I wouldn't want to share you! You are irreplaceable. To my soul mate Mitchell Baird, I thank you for sticking by me every day, through the hard times, the boring times, and (almost) every crazy idea that popped into my head. You are my rock, I couldn't have survived without you. I love you all very much; thank you for loving me in return.

And to everyone else who has had a hand in my life up until this point, I thank you for contributing to something so complicated yet wonderful.

