Systematic review and meta-analysis of the association between complement component 2-3 and factor B polymorphisms and age-related macular degeneration: A HUGE review

Ammarin Thakkinstian

Section for Clinical Epidemiology and Biostatistics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

e-mail: raatk@mahidol.ac.th, Tel: 6622011762, Fax: 6622011284

Mark McEvoy

Lecturer in Genetic Epidemiology, Centre for Clinical Epidemiology & Biostatistics

University of Newcastle, Newcastle, Newcastle, NSW, Australia

e-mail: mark.mcevoy@newcastle.edu.au Gareth J McKay
Centre for Vision and Vascular Sciences, Queen's University of Belast, Northern Ireland, UK
e-mail: g.j.mckay@qub.ac.uk

Usha Chakravarthy
Centre for Vision and Vascular Sciences, Queen's University of Belast, Northern Ireland, UK
e-mail: U.Chakravarthy@qub.ac.uk

Guiliana Silvestri
Centre for Vision and Vascular Sciences, Queen's University of Belast, Northern Ireland, UK
e-mail: G.Silvestri@qub.ac.uk

Xiaoxin Li
Chair of People's Eye Center & Eye Institute, People's Hospital of Peking University
Beijing, China
e-mail: drxiaoxinli@gmail.com

John Attia

Centre for Clinical Epidemiology and Biostatistics, University of Newcastle, Hunter Medical Research Institute, and Department of General Medicine, John Hunter Hospital, Newcastle, Australia. e-mail: john.attia@newcastle.edu.au

Corresponding author: Ammarin Thakkinstian, John Attia

Background

Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world [1-4], accounting for half of all new cases of registered blindness[5]. With an aging population, the burden of AMD is set to grow, with almost 30% of those older than 75 years showing early signs of the disease[1, 6, 7]. The pathological hallmark of the disease is drusen, deposits of protein and lipid, in the retinal pigment epithelium (RPE). This maculopathy progresses to degeneration in 2 forms: geographic atrophy in which there is loss of RPE and photoreceptors, and neovascular AMD in which there is choroidal neovascularisation and hemorrhage.

Since 2005, polymorphic variation in genes underpinning this complex disease have implicated the *ARMS2* locus including *LOC387715*/serine protease *HTRA1* at 10q26[8-12] in addition to several genes involved in the complement pathway. Initial studies implicated variants in the alternative complement pathway genes complement factor H (*CFH*) and complement factor B (*CFB*), with additional independent variants identified in genes encoding classical complement pathway components, such as complement component 2 (*C2*)[13-18, 19] and complement component 3 (*C3*)[20-35]. The *C2* gene, located on 6p21.3, encodes a serum glycoprotein that functions as part of the classical pathway of the complement system involved in innate immunity and inflammation (OMIM#217000) and within which, 2 polymorphisms (rs9332739 G>C and rs547154 G>T), have been implicated. The minor C and T allele frequencies range from 0 to 8.7% for rs9332739 [36] and 2.5% to 11.0% for rs547154 [14, 15, 18, 31]respectively. These polymorphisms may be associated directly with AMD or indirectly through the high level of linkage disequilibrium that exists between *C2* and *CFB*, which is located 500 base pairs downstream on the same chromosome and which contains additional variants that are also highly associated with AMD[15, 18].

The *C3* gene, located on 19p13.3-p13.2 (OMIM+120700), also has 2 polymorphisms highly

associated with AMD and which are reported to be in high LD (r^2=0.85)[27] (rs2230199 C>G and rs1047286 G>A). C3 is an acute phase reactant, involved in increased synthesis of C3 during any inflammatory process. The minor allele frequencies range from 0.8% to 20.6% for rs2230199 [36] and 0 to 23.9% for rs1047286 [36]. The *C3* polymorphisms may contribute to AMD via *CFH*, which acts as a cofactor with C3b inactivator to regulate the activity of C3 convertases [37], or act independently, contributing to AMD disease pathophysiology [38].

We will conduct a systematic review to pool the results of all available population-based association studies between *C2 (*rs547154, rs9332739), *C3* (rs2230199 and rs1047286), and AMD with the following objectives:

- To estimate the prevalence of the minor alleles of *C2* and *C3*
- To ascertain if there are genetic effects on AMD susceptibility, and if so to estimate the magnitude of that gene effect and the possible genetic mode of action
- To assess haplotype effects of the 2 polymorphisms in *C2*, the 2 in *C3*, and between *C2* and *CFB* on AMD

MATERIAL AND METHODS

Search strategy

Studies will be located in Medline and EMBASE databases using PubMed and Elsevier search engines. One reviewer (TA) will locate relevant studies using the search strategy described below.

Search strategy for Medline (PubMed)

(gene OR allele OR polymorphism) AND (macular degeneration) AND (("Complement component 3" OR C3 OR "complement factor 3") OR ("Complement component 2" OR C2 OR "complement factor 2"))

Search strategy for EMBASE (Elsevier)

1. gene
2. allele
3. polymorphism
4. macular degeneration
5. ‘complement component 3’
6. ‘complement factor 3’
7. C3
8. ‘complement component 2’
9. ‘complement factor 2’
10. C2
11. (1 OR 2 OR 3)
12. (5 OR 6 OR 7)
13. (8 OR 9 OR 10)
14. 11 AND 4 AND (12 OR 13)

The reference lists of the retrieved articles will also be reviewed to identify publications on the same topic. Where there are multiple publications from the same study group the most complete and recent results will be used.

Inclusion criteria

Two reviewers (TA and MM) will independently go through all titles or abstracts of those identified studies in order to select the studies to include into the review. Any human population-based association study, regardless of sample size, will be included if it meets the following criteria:

- Genotyped complement component 2 (rs547154 G>T and/or rs9332739 G>C polymorphisms) or Complement component 3 (rs2230199 C>G and/or rs1047286 G>A polymorphisms).

- The outcome is AMD and there are at least two comparison groups, e.g., AMD versus control groups. The AMD is graded as drusen, pigment abnormalities in retinal pigment epithelium, geographic atrophy , and choroidal neovascularization. If AMD grading data are available, early AMD (i.e., drusen and pigment abnormalities in retinal pigment epithelium), geographic atrophy, choroidal neovascularization, and mixed advance AMD (geographic atrophy andchoroidal neovascularization in each eye) will be analyzed separately. These gradings will be collapsed into ”wet” and “dry” AMD, as well as overall AMD groups. Controls are subjects who did not have AMD.

- There are sufficient results for extraction of data, i.e. number of subjects for each genotype in AMD and control groups. Where eligible papers have insufficient information, we will contact authors by e-mail for additional information.

Exclusion criteria

Studies will be excluded from the review for the following reasons:

- Animal study
- Case report
- Family-based study
- Review
- Not AMD
- Not C3 or C2
- Not genetic association studies

- Functional study
- Study in only AMD without having control group
- Methodological study

Data extraction

Summary data for C2 and C3 will be extracted independently and in duplicate by two reviewers (TA & MM) using a standardized data extraction form, appendix I. Covariables such as mean age, percent male, percent smoker, and ethnicity, will also be extracted. Any disagreement will be solved by consensus.

For C2, C3, and CFB, corresponding authors who had reported both C2, both C3, and/or CFB polymorphisms will be contacted to request individual patient data (IPD). This consists of genetic polymorphisms for both C2 or C3 polymorphisms and/or CFB (rs4151667, rs641153, rs2072633), demographic, and clinical variables (e.g., age, gender, smoking, ethnicity, type of AMD cases and AMD grading in each eye, and controls). Data cleaning and checking will be performed separately for each study. Any unclear coding or outlier will be clarified by contact with the authors.

Risk of bias assessment

The quality of studies will be independently assessed by two reviewers (TA & MM) using a risk of bias score for genetic association studies, which is modified based on both traditional epidemiological considerations as well as genetic issues[40-43], see appendix II. The score consists of 5 domains, which are selection bias, information bias, confounding bias, multiple tests & selective reports, and Hardy-Weinberg equilibrium assessment. For selection bias, representativeness of cases and controls, and differential participation in cases and controls are assessed. Ascertainment of diagnosis of AMD and controls, and genotyping methods are assessed for information bias.

Confounding bias such as population stratification, and other confounder effects are considered. The number of polymorphisms that have been studied, adjusting for multiple tests, and the selection of reporting results, are also assessed. Finally, assessing HWE in the control groups of each included study is also considered. Each item will be classified as low/no risk of bias ("yes"), possible/high risk of bias ("no"), or unclear if there is insufficient information to assess ("unclear").

Statistical analysis

Hardy-Weinberg equilibrium (HWE) will be assessed in the control group of each study using an exact test. The disequilibrium coefficient will be also estimated. The analyses will be performed as follows:

i) Pooled allele prevalence

Data in control groups only will be used for pooling allele prevalence. Overall prevalence of minor allele will be pooled for each polymorphism. Heterogeneity will be assessed, and if present, the random effect model will be used for pooling and subgroup analysis by covariable (e.g., ethnicity) will be performed if data is available.

ii) Overall test of genetic association:

The Q test for heterogeneity will be performed for each polymorphism separately for 2 odds ratios (ORs), i.e., *AA* versus *aa* (OR_1), and *Aa* versus *aa* (OR_2) where AA, Aa, aa are common homozygous, heterozygous, and minor homozygous genotypes, respectively. If there is heterogeneity in at least one of these ORs, the cause of heterogeneity will be explored by fitting a covariable (e.g. age, percent male, or percent smoker) in a meta-regression model if the data for these co-variables are available [44-47]. A mixed effects hierarchical model with logit link

function[41] will be applied to determine overall gene effect using the xtmelogit command in STATA. The genotypes will be included in the model as fixed effects, whereas the study will be included as a random effect. A likelihood ratio (LR) test will then be applied to assess whether the gene effect is significant.

iii) Magnitude and genetic model:

Once a gene effect is confirmed, the per-genotype analysis will be used to ascertain the genetic model. The genotype effects will be estimated using the model-free approach [39]. The OR_1 and OR_2 will be estimated using multi-variate meta-analysis with Bayesian methods in which both between and within study variation are taken into account. A parameter lambda (λ), i.e., the ratio of $logOR_2$ versus $logOR_1$ will be calculated to reflect the genetic mode of action as follows: if $\lambda = 0$ then a recessive model is suggested; if $\lambda = 1$ then a dominant model is suggested: if $\lambda = 0.5$ then a co-dominant model; and if λ is greater than 1 or less than 0, then a homozygous or heterosis model is likely.

iv) Inferring haplotype;

Pairwise linkage disequilibrium (LD) coefficient (D', r^2) between polymorphisms within C2 (rs547154 G>T, rs9332739 G>C), within C3, and between C2 (rs547154 G>T, rs9332739 G>C) and CFB (rs4151667, rs641153, rs2072633), will be estimated. If they are highly linked, haplotype frequencies of C2, C3, and CFB polymorphisms will be inferred based on the E-M algorithm using haplologit command in STATA. Odds ratio will then be estimated using profile likelihood. The LR test will be used to test whether the haplotype effect is significant.

Two approaches for handling Hardy-Weinberg disequilibrium (HWD) will be taken. First, sensitivity analyses will be performed by including and excluding studies not in HWE. Second, all studies will be included regardless of HWE and instead adjust for the degree of disequilibrium

using the inbreeding coefficient (F) as described by Trikalinos et al. [48] Briefly, the inbreeding coefficient (F) will be estimated for each study using data in the control group. The predicted genotype frequencies will be estimated [49] and used instead of the observed frequencies in the summary analysis of magnitude and genetic model.

Publication bias will be assessed using the Egger test [50, 51]. Cumulative meta-analysis of the main finding will be performed to assess whether the genetic effects are varied consistently over time. [51-53] Population attributable risk (PAR) for having risk genotypes will be determined. [54, 55] Analyses will be performed using STATA version 11.0 [56] and WinBugs 1.4.2 [57] with normal vague prior distributions for estimation of parameters (i.e., lambda and odds ratio).. The models will be run for a burn-in of 10000 iterations, followed by 50000 iterations for parameter estimates. A *P*-value less than 0.05 will be considered statistically significant, except for tests of heterogeneity where a level of 0.10 is used.

Finally, results of this review will be graded as a level of evidence of genetic association of AMD based on the recommendation of Ioannidis.[43] Three components will be used for grading as follows: how large the frequency of the minor allele is, replication as assessed using degree of heterogeneity I^2, and risk of bias in the meta-analysis. These three items will be graded as A, B, C, which refer to strong (A), moderate (B), and mild (C), respectively. Then, the three components are combined and the evidence is graded as strong (AAA), moderate (2A+1B), or weak evidence (0-1A+ others).

Reference

1. Klein ML, Schultz DW, Edwards A, et al. Age-related macular degeneration. Clinical features in a large family and linkage to chromosome 1q. *Arch Ophthalmol* 1998 Aug;**116**(8): 1082-8.

2. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology* 1995 Oct;**102**(10): 1450-60.

3. Pang CP, Baum L, Chan WM, Lau TC, Poon PM, Lam DS. The apolipoprotein E epsilon4 allele is unlikely to be a major risk factor of age-related macular degeneration in Chinese. *Ophthalmologica* 2000;**214**(4): 289-91.

4. VanNewkirk MR, Nanjan MB, Wang JJ, Mitchell P, Taylor HR, McCarty CA. The prevalence of age-related maculopathy: the visual impairment project. *Ophthalmology* 2000 Aug;**107**(8): 1593-600.

5. Evans J, Wormald R. Is the incidence of registrable age-related macular degeneration increasing? *Br J Ophthalmol* 1996 Jan;**80**(1): 9-14.

6. Schmidt S, Klaver C, Saunders A, et al. A pooled case-control study of the apolipoprotein E (APOE) gene in age-related maculopathy. *Ophthalmic Genet* 2002 Dec;**23**(4): 209-23.

7. Vingerling JR, Dielemans I, Hofman A, et al. The prevalence of age-related maculopathy in the Rotterdam Study. *Ophthalmology* 1995 Feb;**102**(2): 205-10.

8. Conley YP, Jakobsdottir J, Mah T, et al. CFH, ELOVL4, PLEKHA1 and LOC387715 genes and susceptibility to age-related maculopathy: AREDS and CHS cohorts and meta-analyses. *Human molecular genetics* 2006 Nov 1;**15**(21): 3206-18.

9. Despriet DDG, Klaver CCW, Witteman JCM, et al. Complement factor H polymorphism, complement activators, and risk of age-related macular degeneration. *Journal of the American Medical Association* 2006;**296**(3): 301-9.

10. Kaur I, Katta S, Hussain A, et al. Variants in the 10q26 gene cluster (LOC387715 and HTRA1) exhibit enhanced risk of age-related macular degeneration along with CFH in Indian patients. *Invest Ophthalmol Vis Sci* 2008 May;**49**(5): 1771-6.

11. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Human molecular genetics* 2005 Nov 1;**14**(21): 3227-36.

12. Thakkinstian A, Han P, McEvoy M, et al. Systematic review and meta-analysis of the association between complement factor H Y402H polymorphisms and age-related macular degeneration. *Human molecular genetics* 2006 Sep 15;**15**(18): 2784-90.

13. Farwick A, Dasch B, Weber BHF, Pauleikhoff D, Stoll M, Hense HW. Variations in five genes and the severity of age-related macular degeneration: Results from the Muenster aging and retina study. *Eye* 2009;**23**(12): 2238-44.

14. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat Genet* 2006 Apr;**38**(4): 458-62.

15. Jakobsdottir J, Conley YP, Weeks DE, Ferrell RE, Gorin MB. C2 and CFB genes in age-related maculopathy and joint action with CFH and LOC387715 genes. *PLoS One* 2008;**3**(5): e2199.

16. McKay GJ, Silvestri G, Patterson CC, Hogg RE, Chakravarthy U, Hughes AE. Further assessment of the complement component 2 and factor B region associated with age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2009 Feb;**50**(2): 533-9.

17. Richardson AJ, Islam FM, Guymer RH, Baird PN. Analysis of rare variants in the complement component 2 (C2) and factor B (BF) genes refine association for age-related macular degeneration (AMD). *Invest Ophthalmol Vis Sci* 2009 Feb;**50**(2): 540-3.

18. Spencer KL, Hauser MA, Olson LM, et al. Protective effect of complement factor B and complement component 2 variants in age-related macular degeneration. *Human molecular genetics* 2007;**16**(16): 1986-92.

19. Kaur I, Katta S, Reddy RK, et al. The involvement of complement factor B and complement component C2 in an Indian cohort with age-related macular degeneration. *Invest Ophthalmol Vis Sci* Jan;**51**(1): 59-63.

20. Bergeron-Sawitzke J, Gold B, Olsh A, et al. Multilocus analysis of age-related macular degeneration. *Eur J Hum Genet* 2009 Sep;**17**(9): 1190-9.

21. Cui L, Zhou H, Yu J, et al. Noncoding variant in the complement factor H gene and risk of exudative age-related macular degeneration in a Chinese population. *Invest Ophthalmol Vis Sci* Feb;**51**(2): 1116-20.

22. Despriet DD, van Duijn CM, Oostra BA, et al. Complement component C3 and risk of age-related macular degeneration. *Ophthalmology* 2009 Mar;**116**(3): 474-80 e2.

23. Edwards AO, Fridley BL, James KM, Sharma AS, Cunningham JM, Tosakulwong N. Evaluation of clustering and genotype distribution for replication in genome wide association studies: The age-related eye disease study. *PLoS ONE* 2008;**3**(11).

24. Francis PJ, Hamon SC, Ott J, Weleber RG, Klein ML. Polymorphisms in C2, CFB and C3 are associated with progression to advanced age related macular degeneration associated with visual loss. *J Med Genet* 2009 May;**46**(5): 300-7.

25. Goto A, Akahori M, Okamoto H, et al. Genetic analysis of typical wet-type age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese population. *Journal of Ocular Biology, Diseases, and Informatics* 2009: 1-12.

26. Gu J, Pauer GJT, Yue X, et al. Assessing susceptibility to age-related macular degeneration with proteomic and genomic biomarkers. *Molecular and Cellular Proteomics* 2009;**8**(6): 1338-49.

27. Park KH, Fridley BL, Ryu E, Tosakulwong N, Edwards AO. Complement component 3 (C3) haplotypes and risk of advanced age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2009 Jul;**50**(7): 3386-93.

28. Pei XT, Li XX, Bao YZ, et al. Association of c3 gene polymorphisms with neovascular age-related macular degeneration in a chinese population. *Curr Eye Res* 2009 Aug;**34**(8): 615-22.

29. Reynolds R, Hartnett ME, Atkinson JP, Giclas PC, Rosner B, Seddon JM. Plasma complement components and activation fragments: associations with age-related macular degeneration genotypes and phenotypes. *Invest Ophthalmol Vis Sci* 2009 Dec;**50**(12): 5818-27.

30. Scholl HP, Fleckenstein M, Fritsche LG, et al. CFH, C3 and ARMS2 are significant risk loci for susceptibility but not for disease progression of geographic atrophy due to AMD. *PLoS One* 2009;**4**(10): e7418.

31. Scholl HPN, Issa PC, Walier M, et al. Systemic complement activation in age-related macular degeneration. *PLoS ONE* 2008;**3**(7).

32. Seddon JM, Reynolds R, Maller J, Fagerness JA, Daly MJ, Rosner B. Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. *Investigative ophthalmology & visual science* 2009;**50**(5): 2044-53.

33. Seitsonen SP, Onkamo P, Peng G, et al. Multifactor effects and evidence of potential interaction between complement factor HY402H and LOC387715 A69S in age-related macular degeneration. *PLoS ONE* 2008;**3**(12).

34. Spencer KL, Olson LM, Anderson BM, et al. C3 R102G polymorphism increases risk of age-related macular degeneration. *Human molecular genetics* 2008;**17**(12): 1821-4.

35. Yates JRW, Sepp T, Matharu BK, et al. Complement C3 variant and the risk of age-related macular degeneration. *New England Journal of Medicine* 2007;**357**(6): 553-61.

36. .

37. Donoso LA, Kim D, Frost A, Callahan A, Hageman G. The Role of Inflammation in the Pathogenesis of Age-related Macular Degeneration. *Surv Ophthalmol* 2006 Mar-Apr;**51**(2): 137-52.

38. Sivaprasad S, Adewoyin T, Bailey TA, et al. Estimation of systemic complement C3 activity in age-related macular degeneration. *Arch Ophthalmol* 2007 Apr;**125**(4): 515-9.

39. Minelli C, Thompson JR, Abrams KR, Thakkinstian A, Attia J. The choice of a genetic model in the meta-analysis of molecular association studies. *Int J Epidemiol* 2005 Aug 22.

40. Attia J, Thakkinstian A, D'Este C. Meta-analyses of molecular association studies: methodologic lessons for genetic epidemiology. *J Clin Epidemiol* 2003 Apr;**56**(4): 297-303.

41. Thakkinstian A, McEvoy M, Minelli C, et al. Systematic review and meta-analysis of the association between {beta}2-adrenoceptor polymorphisms and asthma: a HuGE review. *Am J Epidemiol* 2005 Aug 1;**162**(3): 201-11.

42. Little J, Higgins J. *The HUGENet HUGE review handbook, version 1.0* 2006.

43. Ioannidis JP, Boffetta P, Little J, et al. Assessment of cumulative evidence on genetic associations: interim guidelines. *Int J Epidemiol* 2008 Feb;**37**(1): 120-32.

44. Thompson JR, Minelli C, Abrams KR, Tobin MD, Riley RD. Meta-analysis of genetic studies using Mendelian randomization--a multivariate approach. *Stat Med* 2005 Jul 30;**24**(14): 2241-54.

45. Thompson SG. Why sources of heterogeneity in meta-analysis should be investigated. *BMJ* 1994;**309**: 1351-5.

46. Thompson SG, Sharp SJ. Explaining heterogeneity in meta-analysis: a comparison of methods. *Stat Med* 1999;**18**: 2693-708.

47. Thompson SG, Smith TC, Sharp SJ. Investigating underlying risk as a source of heterogeneity in meta-analysis. *Stat Med* 1997;**16**: 2741-58.

48. Trikalinos TA, Salanti G, Khoury MJ, Ioannidis JP. Impact of violations and deviations in Hardy-Weinberg equilibrium on postulated gene-disease associations. *Am J Epidemiol* 2006 Feb 15;**163**(4): 300-9.

49. Hernandez JL, Weir BS. A disequilibrium coefficient approach to Hardy-Weinberg testing. *Biometrics* 1989 Mar;**45**(1): 53-70.

50. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997 Sep 13;**315**(7109): 629-34.

51. Egger M, Smith G, Altman D. *Systematic reviews in health care: meta-analysis in context.* Second ed. London: BMJ Publishing Group; 2001.

52. Ioannidis JP, Trikalinos TA. Early extreme contradictory estimates may appear in published research: the Proteus phenomenon in molecular genetics research and randomized trials. *J Clin Epidemiol* 2005 Jun;**58**(6): 543-9.

53. Trikalinos TA, Churchill R, Ferri M, et al. Effect sizes in cumulative meta-analyses of mental health randomized trials evolved over time. *J Clin Epidemiol* 2004 Nov;**57**(11): 1124-30.

54. Hayden KM, Zandi PP, Lyketsos CG, et al. Apolipoprotein E genotype and mortality: findings from the Cache County Study. *JAmGeriatrSoc* 2005 Jun;**53**(6): 935-42.

55. Rossman MD, Thompson B, Frederick M, et al. HLA-DRB1*1101: a significant risk factor for sarcoidosis in blacks and whites. *Am J Hum Genet* 2003 Oct;**73**(4): 720-35.

56. StataCorp. *Stata Statistical Software: Release 11.0*. Collage Station, TX: Stata Corporation; 2009.

57. Spiegelhalter D, Thomas A, Best N, Lunn D. WinBUGS User Manual. 1.4 ed. UK: MRC Biostatistics Unit; 2007.

Appendix I

Data Extraction Form

Association between C2, C3 polymorphisms and age-related macular degeneration

Study ID……..

Reviewer …….

Date of review …….

Authors …….

Year…….……

1. Type of study design

(1) Cohort study

(2) Case-control study

Type of controls ( ) un-matched ( ) matched

(3) Cross-sectional study

2. Type of AMD

( ) drusen ( ) pigment abnormalities in retinal pigment epithelium

( ) Geographic atrophy ( ) choroidal neovascularization ( ) not defined

3. Patient characteristics:

Variable	Case n =	Control n =	total
Age			
Male/female			
Ethnicity			
Smoking			

4. Polymorphisms and outcomes

C2	AMD N=	Control N=	C3	AMD	Control			
rs547154 (IVS10)			rs2230199 (Arg80Gly)*					
G			C					
T			G					
GG			CC					
GT			CG					
TT			GG					
rs9332739 (E318D)			rs1047286 (Pro292Leu)					
G			G					
C			A					
GG			GG					
GC			GA					
CC			AA					

*amino acid (location) data

4. gene-gene Interactions

C2: E318D- IVS10

rs547154 (IVS10)	rs9332739 (E318D)	AMD	Control		
GG	GG				
	GC				
	CC				
GT	GG				
	GC				
	CC				
TT	GG				
	GC				
	CC				

C2-E318D and CFB-L9H

rs9332739 (E318D)	rs4151667 (L9H)	AMD	Control
GG	TT		
	TA		
	AA		
GC	TT		
	TA		
	AA		
CC	TT		
	TA		
	AA		

C2-IVS10 and CFB-R32Q

rs547154 (IVS10)	Rs641153 (R32Q)	AMD	Control		
GG	GG				
	GA				
	AA				
GT	GG				
	GA				
	AA				
TT	GG				
	GA				
	AA				

C2-IVS10 and CFB-IVS17

rs547154 (IVS10)	Rs2072633 (IVS17)	AMD	Control		
GG	GG				
	GA				
	AA				
GT	GG				
	GA				
	AA				
TT	GG				
	GA				
	AA				

Appendix II: Risk of bias assessment for genetic association studies of AMD

Domain	Item	Low risk of bias
Selection bias	Representativeness of cases	
	A. Consecutive/randomly selected from cases population with clearly defined random frame	Yes
	B. Consecutive/randomly selected from cases population without clearly defined random frame or with extensive inclusion criteria	Yes
	C. Spectrum of diseases Select on advance (atrophy or neovascular) or mild AMD	No
	D. Not describe method of selection	
	Representativeness of controls	
	E. Controls were consecutive/randomly drawn from area (ward/community) as cases with the same criteria	Yes
	F. Controls were consecutively/randomly drawn from different areas as cases	No
	G. Not describe	No
	Differential participation in case and control	
	Non-participant rate is small (< 10%) and similar (to rates?) between case and control groups	Yes
	Incomplete participant rates are different	NO
	- Refusal or inability to provide data	
	- Refusal or inability to provide biological specimens	
	- Insufficient amount quality of data/ quality of DNA	
Information bias	Ascertainment of AMD	
	- Clearly described objective criteria of diagnosis of AMD	Yes
	- Not describe/unclear definition	No

	Ascertainment of control	Yes
	- Controls were non-AMD that proved by ocular examination	No
	- Just mentioned that controls were subjects who did not have AMD without ocular examination	No
	- Not describe	
	Ascertainment of genotyping examination	
	- Genotyping done under "blind" condition of case and control specimens	Yes
	- Genotyping of cases & controls were performed together	Yes
	- Genotyping error rate < 5%	Yes
	- Quality control procedure e.g., reanalysis of random specimens, using different genotyping methods for analysis, analysis if replicate sample	Yes
	- Unblind or	No
	- Not mention what was done	No
	- No quality control check	No
Confounding bias	Population stratification	
	- No difference in ethnic origin between cases and controls	Yes
	- Use of controls who were not related to cases	Yes
	- Use of some controls who came from the same family	No
	- Use of genomic controls	No
	- Not report what was done	
	Other confounding bias	Yes
	- Controls for confounding variables (e.g., age, gender, smoking) in analysis	No
	- Not controlled /not mentioned (or, no control/ no mention)	

Multiple testing &	How many polymorphisms have been studied	
	- Adjustment for multiple tests	Yes
Selective reporting (for replication studies)	- Report results of all polymorphisms mentioned in objectives, non-significant or not	Yes
	- Report results of only significant polymorphisms	No
HWE	- HWE in control group	Yes
	- HW disequilibrium in control group	No
	- Not check HWE	No

Yes=low/no risk of bias, No = possible/high risk of bias