1.a. Full Title: CFH polymorphisms and Age-Related Macular Degeneration in Whites and African-Americans.

b. Abbreviated Title (Length 26 characters): CFH and AMD

2. Writing Group (list individual with lead responsibility first):

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal.

Gerald Liew

First author: Gerald Liew, MD, MPH
Address: Center for Vision Research
Westmead Hospital
Westmead NSW 2145
AUSTRALIA

Phone: Tel: +61 2 9845 5551/ Fax: +61 2 9845 8345
Email: gerald_liew@wmi.usyd.edu.au
gerald_liew@yahoo.com.au

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):

Senior author: Tien Wong, MD, PhD
Address: Centre for Eye Research Australia
University of Melbourne
32 Gisborne Street
Melbourne, VIC 3002
AUSTRALIA

Phone: Tel: +61 (3) 99298352 / Fax: +61 (3) 9662 3859,
Email: twong@unimelb.edu.au

Writing group members: Gerald Liew; Ronald Klein; Barbara EK Klein; Mary Frances Cotch; Jie Jin Wang; Ning Cheung; Tien Yin Wong.

3. Timeline:

The objectives of this analysis are to describe the association of CFHY402H polymorphism with early and any (early plus late) age-related macular degeneration (AMD) in whites and African Americans and to determine potential interaction with cardiovascular risk factors, including smoking, body mass index (BMI), lipids, carotid plaque and carotid intima-media thickness (IMT), and inflammatory biomarkers. We will also attempt to replicate recent reports of association of other polymorphisms in CFH and other complement-related genes with early and any AMD. The initial analyses and writing will take place between August and November 2007, and final writing and manuscript submission between December 2007 and May 2008.
4. Rationale:

**CFH polymorphisms are a major Genetic Determinant of AMD**

A major achievement in the last few years was the discovery that variants in the gene encoding complement factor H (CFH) are a key contributor to the risk of age-related macular degeneration (AMD). In white populations, a single copy of the CFHY402H variant is consistently associated with 2 to 3 fold higher risk of late AMD. Other genetic variants in CFH and key complement regulatory proteins may also influence risk of AMD. The CFH gene and five CFH-related genes (CFHR1-5) lie within the regulators of complement activation (RCA) locus on chromosome 1q32, and a number of protective haplotypes at this locus have been identified. Recently a common polymorphism coding a functional variant of the complement 3 protein (C3) was associated with increased risk of late AMD, while genetic variants in complement factor B (CFB) and complement component 2 (CC2) have been reported to be protective against AMD. These results suggest that polymorphisms in CFH, including but not limited to CFHY402H, as well as polymorphisms in genes coding for key complement regulatory proteins (C3, CFB, CC2) may determine a major portion of AMD risk.

**Effect of CFH variants on AMD subtypes is not clear.**

Most studies to date have examined the influence of CFH polymorphisms on late AMD, or the progression of early to late AMD. Few population-based data exist on the effect of CFH polymorphisms on early AMD and specific AMD lesions such as drusen and pigment abnormalities. Some studies have reported that CFHY402H polymorphism may be related more to drusen formation than other aspects of early AMD such as pigment abnormalities. Complement proteins including CFH are a component of drusen, which further emphasizes that CFHY402H polymorphism may be more closely related to drusen than other features of AMD. The ARIC study has randomly sampled population data on over 500 persons with early AMD, drusen and pigmentary abnormalities and provides an opportunity to examine in closer detail the association of CFHY402H polymorphism, as well as other polymorphisms in CFH, C3, CFB and CC2 gene, with these specific early AMD lesions. The ARIC population has another unique advantage in that a subsample (approximately 2000 individuals) of the initial sample were followed up over 10 years, allowing us to examine the association of CFH polymorphisms with progression from early to late AMD over this period. Finally, most epidemiological studies have reported associations of CFH polymorphisms with late AMD, but few have examined if genetic CFH polymorphisms correlate with levels of CFH in their populations. Levels of CFH were assayed in a subsample of ARIC study participants, allowing us to examine in closer detail the genetic pathways from CFH gene, to expression of CFH phenotype, and to final disease onset.

**Racial Differences in Association of CFHY402H and AMD**

The CFHY402H polymorphism has been consistently associated with increased risk of late AMD in white populations, but its role in non-white populations is less clear. Some studies in Japanese and Chinese populations have demonstrated an increased risk of AMD with CFHY402H polymorphism, while other studies have failed to find any associations. These ethnic differences may be due to different prevalence of disease-associated CFH variants in different races. For example, the frequency of CFHY402H polymorphism in Japanese AMD cases is considerably lower than the corresponding frequency in white AMD cases, which may explain in part the lower prevalence of late AMD in Japanese. The association of CFH variants with AMD risk in African-American populations is unknown. A previous report from the ARIC study by some of the same authors as in this proposal found a lower prevalence of early AMD in African-Americans (3.7%) compared to whites (5.6%). Similar findings of lower rates of AMD in African-Americans compared to whites have been reported from a number of studies including the National Health and Nutrition Examination Survey III, the Baltimore Eye Survey, the Barbados Eye Study (Caribbean blacks) and the Multi-Ethnic Study of Atherosclerosis. It is not clear if the lower rates of AMD in African-Americans may be related to lower prevalence of CFH variants in this ethnic group. A recent study...
examined the frequency of the *CFHY402H* polymorphism in different ethnic groups and found similar prevalence rates in whites (0.34) and African-Americans (0.35),
17 suggesting that this particular polymorphism may not be a major factor explaining racial differences in AMD prevalence between blacks and whites. However, this study was not population-based, relied on small numbers (148 whites, 203 African-Americans composed of 75 African Americans and 128 Somalians) and only recruited persons free from AMD and thus may have provided potentially biased estimates of *CFHY402* polymorphism prevalence in blacks. Alternatively, if the prevalence of *CFHY402H* polymorphism in blacks and whites is indeed the same, but the rates of AMD are lower in blacks, this may suggest the possibility of gene-environment interactions where the *CFHY402H* variant may interact with other genes or environmental/systemic factors for which race is a proxy, in expressing the AMD phenotype.
17 This prospect is plausible, as AMD risk has long been known to be vary by ethnicity, and this difference is not explained by varying levels of exposure to risk factors (such as smoking),
12-14,18 suggesting race-specific genetic differences may play a role. The frequencies of different *CFH* variants and *CFH*-related genes can vary considerably by race,
19 further supporting the possibility that the distribution of these genetic factors may partly explain the race-specific differences in AMD risk.

As far as the authors are aware, no studies have specifically examined the prevalence and associations of *CFHY402H* polymorphism, or other single nucleotide polymorphisms (SNPs) in *CFH* gene, in a black population of individuals with and without AMD, largely because of the scarcity of bi- or multiracial population studies with data on genetics and eye diseases. In whites, the population attributable risk (PAR) of *CFHY402H* polymorphism is reported to be up to 50% for late AMD,
11,20,21 although this may be an overestimate as it is derived primarily from case-control rather than population-based studies. The PAR in African-Americans is unknown. The ARIC study thus presents a good opportunity to examine the issue of whether *CFHY402H* modulates early AMD risk to the same extent in both whites and African-Americans, whether this may potentially explain the lower rate of AMD in African-Americans, and to report the PAR of *CFHY402H* polymorphism in African-Americans. We will also examine the distributions and effect of other *CFH* polymorphisms, and variants in *C3*, *CFB* and *CC2* on risk of AMD in whites and African-Americans.

**Association of *CFHY402H* with AMD may be modified by Cardiovascular Risk Factors**

AMD is a complex disease, in which multiple genes and environment interactions may play a role in pathogenesis. Current evidence suggest that genetic factors such as *CFHY402H* (or other genetic variants linked to AMD) on their own are not sufficient to cause AMD,
10 and combination or interaction with environmental systemic factors is required to express the AMD phenotype.
10 There is evidence that the association of *CFHY402H* polymorphism and AMD may be modified by cardiovascular risk factors. Higher BMI, for example, is associated with a pro-inflammatory state,
22 which may contribute to an increased risk of AMD.
9,10 Some case control,
23 cross sectional,
24-26 and prospective studies,
27 have reported associations of higher BMI with AMD, but this has not been confirmed in longitudinal population-based studies.
28 A recent report from the Age-Related Eye Disease Study (AREDS) found that *CFHY402H* polymorphism may interact with higher BMI to increase risk of AMD.
21 Compared to persons without the risk allele, persons with one or two copies of the risk allele and BMI ≥ 25 had odds ratios, OR, of 2.2 (95% confidence interval, CI 1.3-4.0) and 5.9 (3.1-11.4) of developing AMD respectively. In persons with BMI < 25, only those with two copies of the risk allele had increased risk with OR 3.9 (1.7- 9.0), while a single copy did not increase risk. As far as we are aware, this finding has not been confirmed in other populations.

Our group has previously reported from the ARIC study a cross sectional association of carotid plaque with some features of early AMD, (OR for retinal pigment epithelial depigmentation 1.77; 1.18-2.65)
12, although there was no association of early AMD with carotid intima media thickness (IMT) or stiffness.
29 The Rotterdam study
30 reported a similar association of carotid plaque with AMD, with OR of 2.5 (1.14-4.5) and 4.7 (1.8-12.2) depending on the location of the plaque; the Multi-Ethnic Study of Atherosclerosis,
31 however, reported a protective association of echolucent carotid plaque with AMD (OR 0.37; 0.18-0.74) while the Cardiovascular Health Study
32 reported no association of carotid plaque with AMD. These inconsistent results may potentially be due to interaction with CFH, which has been implicated in atherogenesis,
33,34 and found in the atherosclerotic plaques of the carotid and coronary arteries.
35,36
Other cardiovascular risk factors have been associated with AMD in some, but not all, studies. The Beaver Dam Eye Study\textsuperscript{37, 38} and Rotterdam Eye Study\textsuperscript{39} reported higher systolic blood pressure, higher pulse pressure and abnormal lipid levels (decreased low density lipoprotein, LDL, and increased high density lipoprotein, HDL) were associated with greater risk of incident early and late AMD, but these findings were not replicated in longitudinal follow-up in the Blue Mountains Eye Study (decreased HDL was associated with incident AMD)\textsuperscript{40} or cross-sectionally in the ARIC study.\textsuperscript{41}

Inflammatory markers have been associated with AMD in several studies – e.g. cross-sectional association of fibrinogen with AMD in the Blue Mountains Eye study (BMES),\textsuperscript{26} C-reactive protein (CRP) with intermediate and advanced AMD in the Age-Related Eye Diseases Study (AREDS)\textsuperscript{42}. Longitudinal associations of inflammatory markers with AMD have also been reported, such as elevated white cell count with 10-year incident early, but not late, AMD in the BMES\textsuperscript{43} and Beaver Dam Eye Study\textsuperscript{44} and elevated CRP and IL-6 with progression of AMD in the AREDS.\textsuperscript{45} However, the Cardiovascular Health Study was not able to confirm an association of CRP with AMD in elderly individuals.\textsuperscript{46}

A possible reason for these inconsistent findings may be gene-environment/systemic factor interactions. To date only smoking, the major modifiable environmental risk factor for AMD, has been studied for interaction with $CFHY402H$, and the majority of studies have reported no statistically significant interaction, \textsuperscript{9, 20, 21, 47} although the possibility of a lack of study power in some of these studies cannot be completely ruled out. The ARIC study provides the opportunity to examine interactions of $CFHY402H$ polymorphisms with cardiovascular risk factors in a large, randomly sampled population with carefully measured data on cardiovascular risk factors.

The Rotterdam study recently reported that the $CFHY402H$ polymorphism is associated with increased risk of acute myocardial infarction, independent of cardiovascular risk factors (hazard ratio, HR 1.77; 1.23, 2.55). A case control study found a similar association of $CFHY402H$ polymorphism with angiographically confirmed coronary artery disease, with a stronger association for homozygous HH carriers.\textsuperscript{48} However, the Physician’s Health Study failed to find a similar association in men.\textsuperscript{49} These findings are interesting as we have previously found in the ARIC population that AMD is independently associated with increased risk of coronary heart disease (CHD), but only in women (HR 1.58, 95% CI 1.03-2.42).\textsuperscript{50, 51} In that analysis we did not adjust for $CFHY402H$ polymorphism, and we now hypothesize that the AMD-CHD association may be due, in part, to this shared genetic factor.

**Summary and Conclusion.**

The ARIC study has several unique strengths – a large, randomly sampled population; follow-up of participants over 10 years; biracial composition with whites and African-Americans; genetic data on key complement regulatory genes ($CFHY402H$ and 7 other SNPs in $CFH$ gene, common polymorphisms in $C3$, $CFB$ and $CC2$) and enough power to examine some gene-environment interactions with cardiovascular risk factors. We thus propose to examine the association of variants of $CFH$, $C3$, $CFB$ and $CC2$ with early and any AMD, as well as progression of early to late AMD, in whites and African-Americans; and possible interaction of $CFHY402H$ polymorphism with cardiovascular risk factors such as blood pressure, smoking, lipids, BMI, carotid plaque and inflammatory biomarkers. We will also determine if the association of AMD and incident CHD events we previously observed may be explained by the shared presence of the $CFHY402H$ polymorphism.

**5. Main Hypothesis/Study Questions:**

1. To determine the distribution of polymorphisms in key complement regulatory genes ($CFH$ ($CFHY402H$ polymorphism, $CFH$ haplotypes, $C3$, $CFB$ and $CC2$) by race and the associations of these genetic variants with early and any AMD, as well as progression of AMD, in whites and African-Americans.

2. To explore the possible interactions between $CFHY402H$ polymorphism and $CFH$ haplotypes with other AMD risk factors, such as blood pressure, smoking, lipids, BMI, carotid plaque and inflammatory biomarkers.

3. To determine if $CFHY402H$ polymorphism may partly explain the association of AMD with incident CHD events in women observed in the ARIC study.
6. Study power

Assuming a CFHY402H polymorphism prevalence of 0.35 in the whole population, and 500 (early) AMD cases, our proposed study has 83% power at 5% significance level to detect an interaction with race that increases the OR by at least 1.5. For CFHY402H interactions with carotid plaque, assuming that 15% of the population have carotid plaque, our proposed study has 73% power to detect an interaction that increases OR by at least 1.5.

7. Limitations

The ARIC study has approximately 570 cases of early, and 15 cases of late, AMD. Depending on the strength of association of CFHY402H polymorphism with early AMD, this may limit our ability to detect statistical interactions. We will increase power by performing analyses with the combined category of any (early plus late) AMD.

Blood for genotyping was extracted from visit 1, while retinal photography and assessment of AMD was performed at visit 3 (6 years later), at which 12887 out of the original 15792 were re-examined (82%) This may introduce selection bias and we will examine if loss to follow-up varies by CFHY402H and other CFH, C3, CFB, CC2 polymorphism status.

Our analyses of incident AMD over 10 years will be drawn from a subsample of the original visit 3 population that were chosen based on their carotid ultrasound characteristics. The subsample was selected to obtain 60% of baseline participants with high carotid IMT (>85 percentile), with the remaining 40% randomly sampled from the remaining population (<85 percentile). As carotid IMT itself may be associated with AMD, this may introduce selection bias. We will minimize this bias by performing analyses stratified by carotid IMT category, and perform weighted analysis, adjusting for sampling fractions. We will consult closely with the Coordinating Center on this, as the sampling fractions are not straightforward. We will acknowledge this potential bias in our Discussion, and be conservative in our interpretation of the longitudinal findings.

7. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

1. Study design: Cross-sectional and cohort study
2. Inclusion criteria: Participants attending visit 3
3. Exclusion criteria: Exclusion criteria: From participants at ARIC visit 3 (n=12,887), exclude persons who whose race is not black/white, with ungradeable retinal photographs or missing retinal variable at visit 3.
4. Outcomes: AMD variables at baseline (visit 3) and 10-year follow up (visit 5) - early AMD, late AMD, any AMD, soft drusen, pigment abnormalities, retinal pigment epithelium depigmentation, retinal pigment epithelium hyperpigmentation.
5. Study factors: CFHY402H polymorphism status - CC, CT and TT; other SNPs in CFH gene; other complement regulatory genes (C3, CFB, CC2)
6. Additional study factors: body mass index, waist to hip ratio, carotid plaque, carotid intima-media thickness, popliteal artery thickening, carotid arterial stiffness, (all from baseline (visit 3). Inflammatory biomarkers : fibrinogen, white blood cell count, serum albumin. (from visit 1, unless visit 3 data are available
7. Covariates: age, race, center, systolic blood pressure, diastolic blood pressure, hypertension, antihypertensive medication use, diabetes, fasting plasma glucose, diabetic medication use, duration of diabetes, cigarette smoking status (never, past, current), pack-years of smoking, plasma total cholesterol, HDL cholesterol, LDL cholesterol, plasma triglycerides, cholesterol lowering medications, prevalent CHD, von Willebrand factor, factor VIII, ABO blood group (all from visit 3, except last three variables from visit 1).
8. CFH serum/activity levels from subsample assayed by Dr Eric Boerwinkle.
9. Data analysis: We will test for multiplicative statistical interaction using appropriate cross product terms in the logistic regression analyses. We will also compare models using the Akaike’s information criterion to determine which models best describe the joint effect of CFHY402H
polymorphism, BMI and carotid plaque. Haplotype analyses will be performed using PHASE 2.0 program. We will use survival analysis to determine if CFHY402H polymorphism is associated with incident CHD, and if the association is explained by the presence of AMD.

7.a. Will the data be used for non-CVD analysis in this manuscript? ___ Yes  X No

b. If Yes, is the author aware that the file ICTDER01 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used? ___X Yes ___ No
(This file ICTDER01 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ___X Yes ___ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER01 must be used to exclude those with value RES_DNA = “No use/storage DNA”? ___X Yes ___ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://bios.unc.edu/units/cscc/ARIC/stdy/studymem.html ___X Yes ___ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ___ Yes ___ No

b. If yes, is the proposal A. primarily the result of an ancillary study (list number*________) ___ __ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)*__________ __________ __________)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

Reference List


45. Seddon JM, George S, Rosner B, Rifai N. Progression of age-related macular
degeneration: prospective assessment of C-reactive protein, interleukin 6, and other
46. McGwin G, Hall TA, Xie A, Owsley C. The relation between C reactive protein and age
47. 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-
48. Pulido JS, McConnell JP, Lennon RJ et al. Relationship between age-related macular
degeneration-associated variants of complement factor H and LOC387715 with coronary
49. Zee RY, Diehl KA, Ridker PM. Complement factor H Y402H gene polymorphism, C-
reactive protein, and risk of incident myocardial infarction, ischaemic stroke, and venous
50. Wong TY, Tikellis G, Sun C, Klein R, Couper DJ, Sharrett AR. Age-related macular
degeneration and risk of coronary heart disease: the Atherosclerosis Risk in Communities
51. Liew G, Wang JJ, Wong TY. Age-related Macular Degeneration and Heart Disease.
52. North BV, Curtis D, Sham PC. Application of logistic regression to case-control