Mitochondrial DNA Haplogroups and Age-Related Maculopathy

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Objective: To investigate whether mitochondrial haplogroups are associated with age-related maculopathy (ARM).

Methods: We assessed the association between mitochondrial haplogroups and ARM in a population-based sample of 3509 persons aged 49 years or older residing west of Sydney. Retinal photographs of both eyes were taken (1999-2001) and subsequently graded for ARM following the Wisconsin grading system. Genetic analysis for mitochondrial DNA haplogroups was performed. Associations between these genetic markers and risk factors for ARM were assessed.

Results: After adjusting for age, sex, and smoking, haplogroup H was associated with a reduced prevalence of any (early and late) ARM (odds ratio [OR], 0.75; 95% confidence interval [CI], 0.58-0.97), early ARM (OR, 0.75; 95% CI, 0.57-0.98), and large distinct and indistinct soft drusen (OR, 0.70; 95% CI, 0.56-0.89). Haplogroup J was associated with a higher prevalence of large, soft distinct drusen (OR, 1.80; 95% CI, 1.18-2.73). Haplogroup U was associated with an increased prevalence of retinal pigment abnormalities (OR, 1.45; 95% CI, 1.11-1.91).

Conclusions: Our findings of associations between different haplogroup types and prevalent ARM or ARM lesions suggest that these haplogroups may be genetic markers indicative of an individual’s susceptibility to ARM.

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AGE-RELATED MACULOPATHY (ARM) is the most frequent cause of irreversible visual impairment and blindness among older people in Western countries.14 Both the prevalence and severity of ARM increase exponentially with age.6 Apart from age, environmental and genetic factors also contribute to the development of ARM with environmental factors triggering disease in genetically susceptible individuals.7 The identification of “at-risk” older individuals8 genetically predisposed to the development of ARM may permit preventive strategies to reduce environmental causes.

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Y402H variant of complement factor H (CFH) has been associated with AMD in North American and European populations.9-11 It has been proposed that CFH accumulates within drusen and is synthesized by the retinal pigmented epithelium (RPE).11 The

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A genetic basis for the development of age-related macular degeneration (AMD) has recently been demonstrated via genetic variation in a regulator of the alternative complement pathway. The Y402H variant of complement factor H (CFH) has been associated with AMD in North American and European populations.9-11 It has been proposed that CFH accumulates within drusen and is synthesized by the retinal pigmented epithelium (RPE).11 The

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Y402H variant of CFH is located within the binding site of CFH for C-reactive protein (CRP), and CRP has been found to be associated with AMD in some but not all studies.12-15 Although plasma CRP levels are partly determined by genetic variation, no association between genetic variants in CRP and AMD has been demonstrated.16 To our knowledge, a link between the CFH mutation and mitochondrial haplogroups has not been identified. The commonest European mitochondrial haplogroup, mitochondrial DNA (mtDNA) haplogroup H, has recently been shown to be a strong independent predictor of

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outcomes of severe sepsis, suggesting an important link between mitochondrial haplogroups and metabolic reserve in infection and inflammation. The physiological decline of mitochondrial function has been observed in the aging process. Further, one of the most common manifestations of some mitochondrial disease is the presence of retinal pigmentary changes, which are similar to those observed in ARM. Although there are important differences in the pattern of retinal pigmentary abnormalities in certain mitochondrial diseases, particularly MELAS syndrome (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) and MIDD (maternally inherited diabetes and deafness), these conditions sometimes have the appearance of salt-and-pepper retinopathy, typically becoming more prominent with age. Retinal pigment abnormalities changes similar to those observed in ARM have been reported in 75% of patients with the MELAS A3243G mutation. Typically, there are symmetrical, often subtle, areas of depigmentation and pigmentation, predominantly involving the posterior pole but occasionally the midperipheral retina. Large or soft drusen, however, are usually absent. Fluorescein angiography demonstrates mottled hyperfluorescent and hypofluorescent areas indicating multiple window defects at the level of the RPE. We aimed to determine whether mtDNA haplogroups are genetic risk markers for individuals who developed ARM in a representative population of 3509 older persons living in a geographically defined area west of Sydney, Australia.

### METHODS

The Blue Mountains Eye Study (BMES) is a population-based survey of vision and common eye disease in an urban population aged 49 years or older in 2 post-code areas of the Blue Mountains region west of Sydney, Australia. In 1991, we identified 4443 eligible noninstitutionalized permanent residents in a door-to-door census. Of this target population, 3659 persons (82.4%) participated in the BMES baseline survey (1992-1994, BMES I). During 1997-1999, 2335 of 3111 survivors (75.1%) participated in 5-year follow-up examinations (BMES II A). In 1999, a repeat door-to-door census was conducted in the same area; 1174 of 1378 newly eligible residents who had moved into the study area or entered the study age group (85.2%) participated in BMES II B during 1999-2000. This report was derived using data collected from 3509 participants during 1997-2000 (BMES IIA + IIB), who completed a detailed questionnaire and underwent a comprehensive eye examination after pupil dilation after providing written informed consent. The western Sydney and northern Sydney area health service human ethics committees approved the study.

At examination, 30° stereoscopic retinal photographs of the macula and other retinal fields of both eyes were taken as described previously using a Zeiss FF3 fundus camera (Carl Zeiss, Oberkochen, Germany). Photographs were obtained for both eyes in 98% and of at least 1 eye in 99% of participants. Details of the photographic grading performed for ARM lesions were previously reported.

Late-stage ARM was defined to include the 2 late ARM lesions, geographic atrophy, and neovascular age-related macular degeneration as described in the international ARM classification; all late cases were adjudicated by a retinal specialist (P.M.). Early-stage ARM was defined as the absence of late-stage ARM and presence of either large (125 µm in diameter), indistinct soft or reticular drusen or both large, distinct soft drusen and retinal pigment abnormalities (hyperpigmentation or hypopigmentation) within the superimposed grading grid. Cases with only either distinct soft drusen or retinal pigmentary abnormalities were described separately and were not included in early ARM category. “Any ARM” was defined to include early and late ARM cases.

Mitochondrial DNA was available from hair follicles and/or blood in 3393 of 3509 BMES II participants (96.7%). Complete data were available for analysis of ARM in 3302 participants (94.1%) and for analysis of retinal pigmentary abnormalities and drusen after excluding 49 late ARM cases, resulting in 3253 participants (92.7%).

Total DNA was isolated from each subject’s hair follicles and/or blood by standard laboratory techniques. The 10 most commonly found mtDNA haplogroups in the European population (H, I, J, K, M, T, U, V, W, and X) and the African superhaplogroup L were categorized by the presence or absence of the well-defined restriction enzyme recognition sites as determined by polymerase chain reaction/restriction length polymorphism analysis.

Comparison of specific mtDNA haplogroup prevalence in persons with and without signs of ARM was performed using χ² statistics. Logistic regression analyses, adjusting for known ARM risk factors (age, female sex, and current smoking), were performed to assess specific mtDNA haplogroup associations with ARM. Further stratified analyses explored possible interactions between mtDNA haplogroups and these risk factors. Odds ratios (ORs) and 95% confidence intervals (CIs) are presented.

### RESULTS

The 3302 BMES II participants in this study comprised 1877 women (56.8%) and 1425 men (43.2%) with a mean age of 66.4 years (range, 49-98 years) and 66.4 years (range, 49-94 years), respectively. The number of siblings in the study was very low. Only 1.8% of the study population had 2 or more siblings in the study while only 0.3% also had a parent in the study and 0.7% had a parent and a child in the study population. Age-related maculopathy was present in 317 individuals (9.6%) and was classified as early ARM in 268 (8.1%) and late ARM in 49 (1.5%) (Table 1). Retinal pigment abnormalities and soft drusen were present in 460 (14.1%) and 397 of 3253 persons, respectively. Soft drusen were further classified as indistinct in 227 (7%) and distinct in 170 (5.6%) cases.

#### PREVALENCE OF ARM, RETINAL PIGMENTARY ABNORMALITIES, AND DRUSEN

Of the 3302 participants, 3152 (95.6%) could be categorized as one of the 10 recognized European mtDNA haplogroups (H, I, J, K, M, T, U, V, W, and X) or the African superhaplogroup L (Table 1). Haplogroup H was the most frequent (42.9%) followed by haplogroups U
ASSOCIATIONS BETWEEN mtDNA HAPLOGROUPS AND ARM

Known ARM risk factors were included in a basic multivariate logistic regression model, and associations between mtDNA haplogroups and ARM were assessed using this basic model. After adjusting for age, sex, and smoking, haplogroup H was associated with a reduced prevalence of any (early and late) ARM (OR, 0.75; 95% CI, 0.58-0.97) (Table 2). Further analysis dividing the any-ARM group into 2 subgroups, early and late ARM, showed similarly significant associations with early ARM (OR, 0.75; 95% CI, 0.57-0.98) but not with late ARM (OR, 0.85; 95% CI, 0.45-1.55). In subgroup analysis stratified by age, this protective association of haplogroup H and early ARM remained significant in persons aged 70 years or older (OR, 0.70; 95% CI, 0.51-0.96) but not significant in persons younger than 70 years (OR, 0.87; 95% CI, 0.52-1.45).

ASSOCIATIONS BETWEEN mtDNA HAPLOGROUPS AND EARLY ARM LESIONS

Haplogroup H was significantly associated with a reduced prevalence of large soft drusen after adjusting for known ARM risk factors (OR, 0.70; 95% CI, 0.56-0.89) (Table 2). This association was similar for distinct soft drusen prevalence (OR, 0.65; 95% CI, 0.47-0.90) and indistinct soft drusen (OR, 0.77; 95% CI, 0.58-1.04), although the latter was marginally nonsignificant. In subgroup analyses stratified by age, this protective association of haplogroup H and any soft drusen remained significant in persons aged younger than 70 years (OR, 0.56; 95% CI, 0.38-0.83) but was marginally nonsignificant in persons aged 70 years or older (OR, 0.78; 95% CI, 0.59-1.03). Conversely, haplogroup J was borderline nonsignificantly associated with increased prevalence of any soft drusen (OR, 1.35; 95% CI, 0.98-1.88) (Table 2). This association with haplogroup J was only significant for distinct drusen prevalence (OR, 1.80; 95% CI, 1.18-2.73) but not for indistinct drusen prevalence (OR, 0.95; 95% CI, 0.60-1.50). Haplogroup U was associated with an increased prevalence of retinal pigment abnormalities (OR, 1.45; 95% CI, 1.11-1.91). This association was significant only in those younger than 70 years (OR, 1.66; 95% CI, 1.14-2.41) but not in those aged 70 years or older (OR, 1.27; 95% CI, 0.85-1.89). We did not find any statistically significant interactions between smoking and mtDNA haplogroups in associations with ARM.

ASSOCIATIONS BETWEEN mtDNA HAPLOGROUPS AND LATE ARM LESIONS

Haplogroup H was significantly associated with a reduced prevalence of late ARM (OR, 0.75; 95% CI, 0.58-0.97) (Table 2). This association was similar for distinct soft drusen prevalence (OR, 0.65; 95% CI, 0.47-0.90) and indistinct soft drusen (OR, 0.77; 95% CI, 0.58-1.04), although the latter was marginally nonsignificant. In subgroup analyses stratified by age, this protective association of haplogroup H and any soft drusen remained significant in persons aged younger than 70 years (OR, 0.56; 95% CI, 0.38-0.83) but was marginally nonsignificant in persons aged 70 years or older (OR, 0.78; 95% CI, 0.59-1.03). Conversely, haplogroup J was borderline nonsignificantly associated with increased prevalence of any soft drusen (OR, 1.35; 95% CI, 0.98-1.88) (Table 2). This association with haplogroup J was only significant for distinct drusen prevalence (OR, 1.80; 95% CI, 1.18-2.73) but not for indistinct drusen prevalence (OR, 0.95; 95% CI, 0.60-1.50). Haplogroup U was associated with an increased prevalence of retinal pigment abnormalities (OR, 1.45; 95% CI, 1.11-1.91). This association was significant only in those younger than 70 years (OR, 1.66; 95% CI, 1.14-2.41) but not in those aged 70 years or older (OR, 1.27; 95% CI, 0.85-1.89). We did not find any statistically significant interactions between smoking and mtDNA haplogroups in associations with ARM.

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Mitochondria are intracellular organelles that provide energy for the cell. They have a key role in many metabolic processes, including respiration, adenosine triphosphate (ATP) biosynthesis, and inorganic ion homeostasis. Mitochondria are known to contain their own genetic material, mtDNA, which is different from nuclear DNA (nDNA). Abnormalities in either mtDNA or in nuclear genes encoding proteins destined for the mitochondria result in mitochondrial diseases.

However, no significant association was found, likely because of small numbers (n = 49). The protective association of haplogroup H on ARM was also evident for a reduced prevalence of any soft drusen. Conversely, individuals with haplogroup J were 80% more likely than other haplogroups to have distinct soft drusen. Haplogroup U was significantly associated with retinal pigment abnormalities in participants aged younger than 70 years. All observed ARM-haplogroup associations were independent of other common ARM risk factors, including age, female sex, and current smoking.

These findings are compatible with the concept that certain mtDNA haplogroups may cause mild deleterious bioenergetic abnormalities rather than merely represent the presence of “neutral” polymorphisms reflecting different ethnic backgrounds. A number of studies have suggested associations between various mtDNA haplogroups and a variety of medical conditions, including Parkinson disease, Alzheimer disease, occipital stroke, multiple sclerosis, azoospermia, and Leber hereditary optic neuropathy (LHON). Furthermore, mtDNA haplogroups are thought to influence the expression or penetration of disease within affected individuals suffering from LHON. A strong association between smoking and haplogroup J has also been found in the disease expression of LHON.

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In addition to pathogenic mtDNA mutations, there are a number of genetic variations to the mtDNA reference sequence that are not thought to lead to human disease; these variations are referred to as neutral polymorphisms. These neutral polymorphisms are thought to reflect different ethnic backgrounds without causing disease (by definition). Certain neutral polymorphisms have now been classified to indicate a specific mitochondrial haplogroup. In this older Australian population, prevalence of each mtDNA haplogroup was similar to data reported from studies of European and North American populations, suggesting that our results would be applicable to other European-based societies.

Table 2. Associations Between the 5 Most Prevalent mtDNA Haplogroups and Age-Related Maculopathy, Retinal Pigment Abnormalities, and Drusen as Odds Ratios With 95% Confidence Intervals

| Haplogroup | ARM | | | | | | Drusen | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Any | Early | Late | RPAs | | Any | Indistinct | Distinct | | | |
| H | No. (%) | 119 (37.5) | 100 (37.3) | 19 (38.8) | 190 (41.3) | 144 (36.2) | 86 (37.9) | 58 (34.1) | | | |
| OR (95% CI) | 0.75 (0.58-0.97) | 0.75 (0.57-0.98) | 0.85 (0.47-1.55) | 0.90 (0.73-1.11) | 0.70 (0.56-0.88) | 0.77 (0.58-1.04) | 0.65 (0.47-0.90) | | | |
| J | No. (%) | 37 (11.7) | 31 (11.6) | 6 (12.2) | 54 (11.7) | 54 (13.6) | 24 (10.6) | 30 (17.7) | | | |
| OR (95% CI) | 1.06 (0.73-1.56) | 1.07 (0.71-1.62) | 1.01 (0.41-2.49) | 1.09 (0.79-1.50) | 1.35 (0.98-1.88) | 0.95 (0.60-1.50) | 1.80 (1.18-2.73) | | | |
| K | No. (%) | 30 (9.5) | 27 (10.1) | 3 (6.1) | 35 (7.6) | 37 (9.3) | 24 (10.6) | 13 (17.7) | | | |
| OR (95% CI) | 1.15 (0.75-1.77) | 1.20 (0.77-1.88) | 0.74 (0.22-2.52) | 0.88 (0.60-1.29) | 1.11 (0.76-1.64) | 1.28 (0.80-2.05) | 0.92 (0.51-1.65) | | | |
| T | No. (%) | 31 (9.8) | 24 (9.0) | 7 (14.3) | 35 (7.6) | 31 (7.8) | 20 (8.9) | 11 (6.5) | | | |
| OR (95% CI) | 1.17 (0.77-1.79) | 1.05 (0.66-1.66) | 1.80 (0.77-4.23) | 0.84 (0.58-1.23) | 0.87 (0.58-1.31) | 1.02 (0.62-1.69) | 0.71 (0.38-1.33) | | | |
| U | No. (%) | 44 (13.9) | 37 (13.8) | 7 (14.3) | 80 (17.4) | 61 (15.3) | 32 (14.1) | 29 (17.1) | | | |
| OR (95% CI) | 1.11 (0.78-1.59) | 1.10 (0.75-1.62) | 1.13 (0.48-2.62) | 1.45 (1.11-1.91) | 1.26 (0.92-1.71) | 1.14 (0.76-1.72) | 1.35 (0.89-2.05) | | | |

Abbreviations: ARM, age-related maculopathy; CI, confidence interval; mtDNA, mitochondrial DNA; OR, odds ratio; RPAs, retinal pigment abnormalities.

4Controlling for age, female sex, and current smoking.
which places a huge oxidative burden on the cells. Although the RPE has evolved effective defenses against oxidative damage, with increasing age the RPE antioxidative capability appears to be reduced.\textsuperscript{45,47} The susceptibility of mtDNA to oxidative damage in human RPE cells, together with the age-related decrease of the cellular antioxidative system, provides the rationale for a mitochondria-based model of AMD.\textsuperscript{45}

In conclusion, these findings are compatible with the hypothesis that mtDNA may alter an individual's susceptibility to ARM, indicated by its associations with ARM and ARM lesions, including soft drusen and retinal pigment abnormalities. Our findings of a significant protective association between haplogroup H and ARM or soft drusen, between haplogroup U and an increased prevalence of pigment abnormalities, and between haplogroup J and an increased prevalence of distinct drusen suggest that these haplogroups may be genetic markers of ARM susceptibility in addition to other identified genetic markers.

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