Mitochondrial Haplogroups and Control Region Polymorphisms in Age-Related Macular Degeneration: A Case-Control Study

Edith E. Mueller¹, Elena Schauer¹, Susanne M. Brunner¹, Waltraud Eder¹, Johannes A. Mayr¹, Stefan F. Egger², Christian Nischler², Hannes Oberkofler³, Herbert A. Reitsamer², Wolfgang Patsch³, Wolfgang Sperl¹, Barbara Kofler¹

¹ Research Program for Receptor Biochemistry and Tumor Metabolism, Department of Pediatrics, Paracelsus Medical University, Salzburg, Austria; ² Department of Ophthalmology, Paracelsus Medical University, Salzburg, Austria; ³ Department of Laboratory Medicine, Paracelsus Medical University, Salzburg, Austria

Abstract

**Background:** Onset and development of the multifactorial disease age-related macular degeneration (AMD) are highly interrelated with mitochondrial functions such as energy production and free radical turnover. Mitochondrial dysfunction and overproduction of reactive oxygen species may contribute to destruction of the retinal pigment epithelium, retinal atrophy and choroidal neovascularization, leading to AMD. Consequently, polymorphisms of the mitochondrial genome (mtDNA) are postulated to be susceptibility factors for this disease. Previous studies from Australia and the United States detected associations of mitochondrial haplogroups with AMD. The aim of the present study was to test these associations in Middle European Caucasians.

**Methodology/Principal Findings:** Mitochondrial haplogroups (combinations of mtDNA polymorphisms) and mitochondrial CR polymorphisms were analyzed in 200 patients with wet AMD (choroidal neovascularization, CNV), in 66 patients with dry AMD, and in 385 controls from Austria by means of multiplex primer extension analysis and sequencing, respectively. In patients with CNV, haplogroup H was found to be significantly less frequent compared to controls, and haplogroup J showed a trend toward a higher frequency compared to controls. Five CR polymorphisms were found to differ significantly in the two study populations compared to controls, and all, except one (T152C), are linked to those haplogroups.

**Conclusions/Significance:** It can be concluded that haplogroup J is a risk factor for AMD, whereas haplogroup H seems to be protective for AMD.

Introduction

In the western world, age-related macular degeneration (AMD) is the most frequent cause of visual loss in people aged 50 or older [1]. The incidence of AMD is low in persons younger than 50 years (0.05%), but climbs to 11.8% in persons over the age of 80 [2]. AMD affects the macula, a retinal region containing the highest density of photoreceptors and generating central high-resolution visual acuity. In AMD, the presence of so-called drusen, deposits of acellular debris in between the retinal pigment epithelium and the Bruch's membrane, localized posterior to the photoreceptors, is the first characteristic sign of the disease. A few small drusen may appear in the retina of individuals over 50 years of age without relation to AMD. An excess of drusen and/or medium sized or large drusen, however, may lead to destruction of the retinal pigment epithelium and, in combination with inflammatory processes, cause atrophy of the retina and mild visual impairment. Subsequent retinal atrophy reaching further into the center of the macula (dry AMD), and also choroidal neovascularization (CNV, wet AMD) with augmented vascular permeability and fragility resulting in retinal edema and/or hemorrhage, may cause visual loss [1].

AMD is a multifactorial disease, where several risk factors and genetic variants may act together, resulting in disease development. Besides age, known risk factors are amongst others smoking and white race [1]. Nuclear encoded genetic susceptibility factors for AMD include the polymorphism Tyr402His in the complement factor H (CFH) gene, as well as the Ala69Ser polymorphism in the age-related maculopathy susceptibility 2 gene (ARMS2) [1,3,4].

The retinal pigment epithelium, being metabolically very active, comprises a high number of mitochondria [5]. In a process called oxidative phosphorylation (OXPHOS), these organelles produce most of the cellular energy in the form of ATP, as well as reactive oxygen species (ROS). Thirteen subunits of the OXPHOS enzymes...
are encoded by the mitochondrial genome (mtDNA) [6]. ROS, including those produced in mitochondria by the electron transport chain, preferentially damage mtDNA. In turn, damaged mtDNA induces mitochondrial dysfunction with disturbed OXPHOS and higher production of ROS, initiating a vicious circle [7].

The retinal pigment epithelium is especially susceptible to mitochondrial dysfunction and ROS damage and, being a post-mitotic tissue, does not regenerate, and thus accumulates mtDNA somatic mutations [8,9]. A higher prevalence of AMD in older people and the progressive nature of the disease, as well as the high susceptibility of the retina to oxidative stress, hint at mitochondrial involvement in the progress of AMD.

Interestingly, some patients suffering from mitochondrial diseases caused by mutations or deletions of mtDNA, such as Leber’s hereditary optic neuropathy (LHON) or Kearns-Sayre syndrome, have been shown to exhibit retinal pigmentary changes resembling features of the early AMD phenotype [10,11].

Neutral polymorphisms of mtDNA or combinations of these, defined as mtDNA haplogroups, might also contribute to AMD onset or progression. Mitochondrial haplogroups (H, J, U and T) and polymorphisms (73, 4917 and 16126, among others) have already been detected to be associated with AMD in case-control studies from the United States (US) and Australia [12–15]. In epidemiological studies it is important to obtain repetitive results in different studies and different geographical regions. This is of high relevance for interpretation of such data, in order to avoid the influence of possible confounding factors that might bias the result of one particular study. In studies analyzing mtDNA, repetitive results are even more crucial, as haplogroup distributions can vary between populations within only small regional distances [16].

Hence, the aim of the present study was to confirm associations between mtDNA haplogroups and AMD that were obtained in US and Australian populations, in Middle-European Caucasians.

**Results**

Mitochondrial haplogroups H and J are associated with CNV

In the present case-control study, haplogroups and polymorphisms of the non-coding CR of mtDNA were analyzed in patients with wet AMD (CNV) and dry AMD and compared to those in controls. Clinical characteristics of the study groups are presented in Table 1.

The frequencies of mitochondrial haplogroups and CR polymorphisms in the control group were very similar to those previously reported in a large control group from Salzburg [17–20]. In patients with CNV, haplogroup H was found at a significantly lower frequency than in the control group [36.0% vs. 44.9%, p = 0.038, OR 0.69 (0.5–1.0); after adjustment for age and sex: p = 0.035, OR 0.68 (0.5–1.0)] (Table 2).

**Table 1.** Characteristics of the study populations.

|                     | Patients with CNV<sup>a</sup> | Patients with dry AMD<sup>b</sup> | Control group |
|---------------------|-------------------------------|----------------------------------|---------------|
|                     | n = 200                       | n = 66                           | n = 385       |
| Mean (SD<sup>c</sup>) age (years) at diagnosis | 77.2 (8.4)                    | 80.9 (6.0)                      | 73.6 (9.5)    |
| Male (%)            | 34.0                          | 42.4                            | 49.9          |

<sup>a</sup>CNV = choroidal neovascularization.
<sup>b</sup>AMD = age-related macular degeneration.
<sup>c</sup>SD = standard deviation.

**Table 2.** Frequencies (%) of mitochondrial haplogroups in Caucasian patients with CNV and in controls.

|                     | Patients with CNV<sup>a</sup> | Control group | P-value<sup>b</sup> |
|---------------------|-------------------------------|---------------|---------------------|
|                     | n = 200                       | n = 385       |                     |
| H                   | 36.0                          | 44.9          | 0.038               |
| U                   | 18.0                          | 15.9          | 0.506               |
| J                   | 15.0                          | 9.6           | 0.052               |
| T                   | 10.5                          | 7.5           | 0.223               |
| K                   | 5.5                           | 3.9           | 0.372               |
| W                   | 0.5                           | 2.3           | 0.176               |
| V                   | 2.0                           | 3.4           | 0.347               |
| I                   | 2.0                           | 0.8           | 0.238               |
| X                   | 2.0                           | 1.6           | 0.742               |
| Others<sup>c</sup>  | 8.5                           | 10.1          | 0.525               |

<sup>a</sup>CNV = choroidal neovascularization.
<sup>b</sup>P-value: Pearson chi-square or Fisher’s exact test.
<sup>c</sup>Haplogroups that could not be assigned to one of the nine major European haplogroups by the SNP combination.

doi:10.1371/journal.pone.0030874.t002

**Table 3.** Frequencies (%) of mitochondrial haplogroups in Caucasian patients with dry AMD and in controls.

|                     | Patients with dry AMD<sup>a</sup> | Control group | P-value<sup>b</sup> |
|---------------------|-------------------------------|---------------|---------------------|
|                     | n = 66                        | n = 385       |                     |
| H                   | 48.5                          | 44.9          | 0.593               |
| U                   | 13.6                          | 15.9          | 0.647               |
| J                   | 7.6                           | 9.6           | 0.599               |
| T                   | 9.1                           | 7.5           | 0.662               |
| K                   | 3.0                           | 3.9           | 1.000               |
| W                   | 6.1                           | 2.3           | 0.107               |
| V                   | 3.0                           | 3.4           | 1.000               |
| I                   | 0.0                           | 0.8           | 1.000               |
| X                   | 4.5                           | 1.6           | 0.131               |
| Others<sup>c</sup>  | 4.6                           | 10.1          | 0.149               |

<sup>a</sup>AMD = age-related macular degeneration.
<sup>b</sup>P-value: Pearson chi-square or Fisher’s exact test.
<sup>c</sup>Haplogroups that could not be assigned to one of the nine major European haplogroups by the SNP combination.

doi:10.1371/journal.pone.0030874.t003
Moreover, haplogroup J was more frequent in patients with CNV compared to the control group [15.0% vs. 9.6%, p = 0.052, OR 1.66 (1.0–2.8); after adjustment for age and sex: p = 0.048, OR 1.72 (1.0–2.9)] (Table 2).

When haplogroup frequencies were compared between patients with dry AMD and the controls, no significant differences were found (Table 3).

Age-related macular degeneration and mitochondrial CR polymorphisms

The mitochondrial CR was sequenced and analyzed between nucleotide position 16038 and 569. All polymorphisms and their frequencies and the results of comparisons between patients with CNV and controls as well as between patients with dry AMD and controls are listed in Table S1 and Table S2, respectively. CR polymorphisms with a frequency higher than 5% are listed in Table 4 and Table 5. The CR polymorphism T152C was found to be present at a significantly higher frequency in patients with CNV compared to controls. An additional four CR polymorphisms were significantly more frequent in patients with CNV compared to controls (Table 4). However, three of these are linked to mitochondrial haplogroup J [C16069T (J), T16126C (JT), C295T (J)] [21].

When haplogroup frequencies were compared between patients with dry AMD and the controls, no significant differences were found (Table 3).

Table 4. Frequencies (%) of control region polymorphisms >5% in patients with CNV and in controls as well as the corresponding odds ratios and 95% confidence intervals.

| Polymorphism in mtDNA* control region | Frequency in patients with CNVa | Frequency in control group | P-valueb | Odds ratio (95% CI)c | P-valuef |
|---------------------------------------|-------------------------------|---------------------------|---------|---------------------|---------|
|                                       | n = 200                       | n = 385                   |         |                     |         |
| C16069T                               | 16.00                         | 10.13                     | 0.039   | 1.69 (1.0–2.8)      | 0.038   |
| T16093C                               | 9.00                          | 8.31                      | 0.778   |                     |         |
| T16126C                               | 28.50                         | 18.70                     | 0.007   | 1.73 (1.2–2.6)      | 0.006   |
| T16189C                               | 14.00                         | 11.17                     | 0.320   |                     |         |
| C16223T                               | 6.50                          | 7.01                      | 0.816   |                     |         |
| C16270T                               | 9.50                          | 5.71                      | 0.089   |                     |         |
| C16294T                               | 11.50                         | 9.61                      | 0.475   |                     |         |
| C16296T                               | 6.00                          | 6.49                      | 0.816   |                     |         |
| T16304C                               | 8.50                          | 9.87                      | 0.590   |                     |         |
| T16311C                               | 14.00                         | 9.87                      | 0.134   |                     |         |
| T16356C                               | 5.50                          | 7.27                      | 0.415   |                     |         |
| T16362C                               | 7.50                          | 6.49                      | 0.647   |                     |         |
| T16519C                               | 63.00                         | 65.45                     | 0.556   |                     |         |
| A73G                                  | 62.50                         | 51.43                     | 0.011   | 1.57 (1.1–2.2)      | 0.012   |
| T146C                                 | 12.50                         | 8.05                      | 0.083   |                     |         |
| C150T                                 | 12.50                         | 10.13                     | 0.384   |                     |         |
| T152C                                 | 28.50                         | 20.78                     | 0.036   | 1.52 (1.0–2.3)      | 0.014   |
| G185A                                 | 8.00                          | 5.71                      | 0.287   |                     |         |
| T195C                                 | 18.50                         | 17.92                     | 0.863   |                     |         |
| G228A                                 | 8.50                          | 5.97                      | 0.251   |                     |         |
| A263G                                 | 98.50                         | 98.44                     | 1.000   |                     |         |
| C295T                                 | 16.50                         | 10.39                     | 0.034   | 1.70 (1.0–2.8)      | 0.035   |
| A302InsCC                             | 43.50                         | 36.62                     | 0.106   |                     |         |
| A302InsC                              | 9.00                          | 13.25                     | 0.131   |                     |         |
| T310InsC                              | 94.50                         | 94.03                     | 0.816   |                     |         |
| C462T                                 | 11.00                         | 6.75                      | 0.076   |                     |         |
| T489C                                 | 16.50                         | 10.91                     | 0.055   |                     |         |
| CAS14/515Del                           | 10.50                         | 10.13                     | 0.889   |                     |         |

*mtDNA = mitochondrial DNA.

CNV = choroidal neovascularisation.

n: number of individuals with the respective polymorphism.

P-value: Pearson chi-square or Fisher’s exact test.

CI = confidence interval.

fadjusted for age and sex by logistic regression analysis.

doi:10.1371/journal.pone.0030874.t004
Twenty-six CR polymorphisms were found with a frequency higher than 5% in both controls and patients with dry AMD. Of these, T195C had a significantly higher frequency in patients with dry AMD compared to controls [33.3% vs. 17.9%, p = 0.004, OR = 2.29 (1.3–4.1); after adjustment for age and sex: p = 0.01, OR = 2.30 (1.3–4.2)] (Table 5).

Discussion

Studies have shown that oxidative stress plays a causative role in the pathogenesis of AMD, and that mitochondria are instrumental in this etiology. For example, Imamura et al. observed in the retinas of Sod1<sup>−/−</sup> mice, which lack the antioxidant enzyme Cu, Zn-superoxide dismutase (SOD1), elevated levels of oxidative damage to DNA and protein as well as development of drusen and CNV with age, signs shared by human AMD [23]. Vives-Bauza et al. reported that a combination of mild OXPHOS defects, caused by the mtDNA T8993G point mutation, together with low levels of the autofluorescent constituent of lipofuscin, A2E, induced a reduction in the phagocytic competence of the retinal pigment epithelium. The combination of low levels of A2E and more severe OXPHOS defects induced retinal pigment epithelium cell death [9]. All these facts support the conclusion that mtDNA variation contributes to the complex etiology of AMD.

Accordingly, in the present study we observed a higher prevalence of mitochondrial haplogroup J and a significantly lower prevalence of haplogroup H in patients with CNV compared to the control group. That the CR polymorphisms linked to haplogroups J and H also showed positive and negative associations, respectively, with CNV further supports the haplogroup–CNV association.

The CR polymorphism T152C, although it is not associated with any particular haplogroup, was also present at a higher frequency in patients with CNV compared to controls. For dry AMD, only one significant association was found, namely a higher frequency of T195C in patients compared to controls. The study group of patients with dry AMD (n = 66) was small, and also no data were available according to a classification into early, intermediate or advanced AMD. Hence, these results should be considered tentative. Associations of T152C and T195C have

| Polymorphism in mtDNA<sup>a</sup> control region | Frequency in patients with dry AMD<sup>b</sup> | n<sup>c</sup> | Frequency in control group | n<sup>c</sup> | P-value<sup>d</sup> |
|-----------------------------------------------|-----------------------------------------------|----------|--------------------------|----------|-----------------|
| C16069T                                       | 9.09                                          | 6        | 10.13                    | 39       | 0.795           |
| T16093C                                       | 7.58                                          | 5        | 8.31                     | 32       | 0.840           |
| T16126C                                       | 16.67                                         | 11       | 18.70                    | 72       | 0.694           |
| T16189C                                       | 13.64                                         | 9        | 11.17                    | 43       | 0.562           |
| C16223T                                       | 12.12                                         | 8        | 7.01                     | 27       | 0.152           |
| C16294T                                       | 9.09                                          | 6        | 9.61                     | 37       | 0.894           |
| T16298C                                       | 6.06                                          | 4        | 5.97                     | 23       | 1.000           |
| T16304C                                       | 9.09                                          | 6        | 9.87                     | 38       | 0.844           |
| T16311C                                       | 12.12                                         | 8        | 9.87                     | 38       | 0.577           |
| T16356C                                       | 7.58                                          | 5        | 7.27                     | 28       | 1.000           |
| T16362C                                       | 6.06                                          | 4        | 6.49                     | 25       | 1.000           |
| T16519C                                       | 75.76                                         | 50       | 65.45                    | 252      | 0.100           |
| A73G                                          | 54.55                                         | 36       | 51.43                    | 198      | 0.640           |
| T146C                                         | 7.58                                          | 5        | 8.05                     | 31       | 0.895           |
| C150T                                         | 12.12                                         | 8        | 10.13                    | 39       | 0.625           |
| T152C                                         | 24.24                                         | 16       | 20.78                    | 80       | 0.525           |
| T195C                                         | 33.33                                         | 22       | 17.92                    | 69       | 0.004           |
| A263G                                         | 33.33                                         | 24       | 36.62                    | 25       | 0.481           |
| C295T                                         | 5.80                                          | 3        | 8.05                     | 31       | 0.481           |
| A302InsC                                      | 10.61                                         | 7        | 13.25                    | 51       | 0.554           |
| T310insC                                      | 9.39                                          | 62       | 94.03                    | 362      | 1.000           |
| T489C                                         | 7.58                                          | 5        | 10.91                    | 42       | 0.413           |
| G499A                                         | 7.58                                          | 5        | 6.23                     | 24       | 0.596           |
| G513insCA                                     | 6.06                                          | 4        | 5.97                     | 23       | 1.000           |
| CA514/515Del                                   | 7.58                                          | 5        | 10.13                    | 39       | 0.518           |

<sup>a</sup>mtDNA = mitochondrial DNA.
<sup>b</sup>AMD = age-related macular degeneration.
<sup>c</sup>n: number of individuals with the respective polymorphism.
<sup>d</sup>P-value: Pearson chi-square or Fisher's exact test.

doi:10.1371/journal.pone.0030874.t005
been reported, with breast cancer in Tunisian women (T152C – weak protective effect) [24] and with early childhood bronchitis (T195C – increased risk) [25]. The mitochondrial CR polymorphisms T152C and T195C are mutational hotspots [21,26], and are found on the background of several haplogroups.

Lack of Bonferroni correction might be considered to be a limitation of the present study. However, we believe that Bonferroni correction is not the adequate method for our type of analysis, because our study was intended to be a confirmation study and because CR polymorphisms that showed significantly different frequencies between controls and patients are strongly linked to haplogroups H and J.

Jones et al. compared mtDNA haplogroup frequencies in 317 patients with AMD to those of 2905 controls from Australia. After adjustment for age, sex and smoking they found haplogroup H to be at a reduced prevalence in the AMD group compared to controls, very similar to the results obtained in the present study (Table 6). Additionally, subjects with haplogroup J were found to have a higher risk of developing large, soft, distinct drusen [12]. Unfortunately, in our study no data concerning drusen development were available.

In 560 Caucasians from the US, Canter et al. detected the mitochondrial haplogroup T-linked polymorphism A4917G to be significantly associated with AMD (Table 6) [13]. In our study, we were not able to find a significant association with this polymorphism, although we did observe a tendency toward a higher frequency of A4917G (11.5% vs. 8.3%, data not shown) as well as of mitochondrial haplogroup T (10.5% vs. 7.5%) in patients with CNV compared to controls (Table 2 and Table 6).

A4917G and the CR polymorphisms C16069T and T16126C, which are linked to haplogroup J (C16069T) and the haplogroup cluster JT (T16126C), were found to be associated with AMD in a case-control study (73 controls and 81 patients) from the US [14]. Moreover, SanGiovanni et al. found a significant association of A4917G and the CR positions 73A and T16126C with advanced AMD in 314 (215 cases and 99 controls) non-Hispanic US and Australian subjects (Table 6) [15].

Our study adds to these previous studies from the US and Australia in providing corroborative data for a population from a geographically well-defined and distinct region of Middle Europe. Combining all the results, the conclusion can be drawn that mtDNA haplogroups J and T are risk factors, and that mtDNA haplogroup H is a protective factor, for AMD in Caucasian populations.

Methods

Ethics Statement

Before entering the study, subjects gave written informed consent, and anonymity of the patients was assured. The study was performed in accordance with the National Gene Technology Act of Austria and followed the Declaration of Helsinki. The study was approved by the Local Province of Salzburg Ethics Committee (“Ethikkommission für das Bundesland Salzburg, Amt der Salzburger Landesregierung, Abteilung 9 Gesundheit und Sport”).

Patients and control subjects

In this case-control study we included a total of 651 Caucasian subjects. All participants were enrolled in the Department of Ophthalmology, University Hospital Salzburg, Paracelsus Medical University. Blood samples from patients and controls were collected from August 2006 to October 2008. Recruitment of patients and controls was done as follows. Each patient diagnosed for CNV was asked if he agreed to participate in the study. Controls were mainly subjects who were kept as an inpatient for cataract surgery. Here, subjects were asked for agreement sporadically. Patients with dry AMD were also recruited sporadically. Sixty-six patients were diagnosed with dry AMD, 200 patients with CNV, and a control group of 385 subjects did not show any signs of AMD (i.e. no

### Table 6. Comparison of age-related macular degeneration case-control studies in the literature with the present study.

| MtDNA haplogroup** or polymorphism | Haplogroup | Frequency (%) in cases | Frequency (%) in controls | P-value* | Odds ratio (95% CI)** | References |
|-----------------------------------|------------|------------------------|---------------------------|----------|----------------------|------------|
| H                                 |            | 37.5                   | 43.5                      | not shown| 0.75 (0.6–1.0)       | [12]       |
|                                   |            | 36.0                   | 44.9                      | 0.035    | 0.68 (0.5–1.0)       | Present study |
| 4917G                             | T          | 15.4                   | 9.0                       | 0.011    | 2.16 (1.2–3.9)       | [13]       |
|                                   |            | 12.2                   | 2.7                       | 0.020    | 5.00 (1.1–23.6)      | [14]       |
|                                   |            | 16.3                   | 4.0                       | 0.001    | 6.15 (2.0–18.5)      | [15]       |
|                                   |            | 11.5                   | 8.3                       | 0.143    | 1.54 (0.9–2.8)       | Present study |
|                                   |            | 10.5                   | 7.5                       | 0.133    | 1.59 (0.9–2.9)       | Present study |
| 73G                               | non-HV,H,V | 62.5                   | 51.4                      | 0.012    | 1.58 (1.1–2.3)       | Present study |
| 73A                               | HV,H,V     | 35.8                   | 48.5                      | 0.030    | 0.58 (0.4–1.0)       | [15]       |
| 16069T                            | J          | 9.9                    | 4.1                       | 0.055    | 3.89 (0.8–19.0)      | [14]       |
|                                   |            | 16.0                   | 10.1                      | 0.038    | 1.74 (1.0–2.9)       | Present study |
| 16126C                            | JT         | 24.7                   | 8.2                       | 0.004    | 3.66 (1.4–9.7)       | [14]       |
|                                   |            | 27.0                   | 13.1                      | 0.007    | 2.51 (1.3–4.9)       | [15]       |
|                                   |            | 28.5                   | 18.7                      | 0.006    | 1.79 (1.2–2.7)       | Present study |

*mtDNA = mitochondrial DNA.

*According to PhyloTree.org [21].

*P-values: present study: adjusted for age and sex, Jones et al. [12]: adjusted for age, sex and current smoking, Canter et al. [13]: adjusted for sex and three nuclear polymorphisms (CFH-Complement Factor H gene, rs1061170; LOC387715, rs10490924; APOE, ApoE2 allele). Udar et al. [14]: no adjustment. SanGiovanni et al. [15]: adjusted for age, sex and smoking.

*CI = confidence interval.

doi:10.1371/journal.pone.0030874.t006
Mitochondrial DNA analysis

Mitochondrial haplogroups were assessed as described before [27]. Haplogroups that could not be assigned to one of the nine major European haplogroups by their single nucleotide polymorphism (SNP) combination were designated as “others”.

CR sequences were analyzed between nucleotide positions 16038 and 569. Polymerase chain reaction and sequencing was performed as described previously [27]; however, the primer 15997f: 5’-CACCATTTAGACCCCAAAGCT-3’ was used instead of 16098f.

Statistical analysis

Frequencies of all mitochondrial haplogroups and CR polymorphisms were tested for independence for the disease using Pearson chi-square statistics and Fisher’s exact test as appropriate. Only haplogroups and polymorphisms with a frequency higher than 5% in both study groups were subjected to further statistical analysis. Associations were adjusted for age and sex using logistic regression analysis. A p-value <0.05 was considered statistically significant. All analyses were performed using PASW statistics 10.0 (SPSS GmbH, Germany).

Supporting Information

Table S1 Frequencies (%) of control region (CR) polymorphisms in patients with CNV and in controls as well as the corresponding odds ratios and 95% confidence intervals.

Table S2 Frequencies (%) of control region (CR) polymorphisms in patients with dry AMD and in controls as well as the corresponding odds ratios and 95% confidence intervals.

Author Contributions

Conceived and designed the experiments: SFE CN HO HAR WP WS BK. Performed the experiments: ES SBM. Analyzed the data: EEM ES SMB WE BK. Contributed reagents/materials/analysis tools: SFE CN HO HAR WP. Wrote the paper: EEM BK. Provided technical support: JAM.

References

1. Jager RD, Meiler WF, Miller JW (2008) Age-related macular degeneration. N Engl J Med 358: 2606–2617.
2. de Jong PT (2006) Age-related macular degeneration. N Engl J Med 355: 1474–1485.
3. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, et al. (2005) Complement factor H polymorphism in age-related macular degeneration. Science 308: 385–389.
4. Rivera A, Fisher SA, Frische LG, Keilhauer CN, Lichtner P, et al. (2005) Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. Hum Mol Genet 14: 3227–3236.
5. Jarrett SG, Lin H, Godley BF, Boulton ME (2008) Mitochondrial DNA damage and its potential role in retinal degeneration. Prog Retin Eye Res 27: 596–607.
6. Wallace DC (1999) Mitochondrial diseases in man and mouse. Science 283: 1432–1438.
7. Liang FQ, Godley BF (2003) Oxidative stress-induced mitochondrial DNA damage in human retinal pigment epithelial cells: a possible mechanism for RPE aging and age-related macular degeneration. Exp Eye Res 76: 397–403.
8. King A, Grotthjeb E, Brooks DG, Murphy MP, Dunmore J (2004) Mitochondria-derived reactive oxygen species mediate blue light-induced death of retinal pigment epithelial cells. Photochem Photobiol 79: 470–475.
9. Vivès-Bauza C, Anand M, Shirazi AK, Magrane J, Gao J, et al. (2008) The age lipid A2E and mitochondrial dysfunction synergistically impair phagocytosis by retinal pigment epithelial cells. J Biol Chem 283: 24770–24778.
10. Sue CM, Mitchell P, Ciminus DS, Moshegov G, Byrne E, et al. (1997) Pigmentary retinopathy associated with the mitochondrial DNA 3243 point mutation. Neurology 49: 1013–1017.
11. Iashkih Y, Nakagawa M, Ohba N, Kamimura K, Sadoya Y, et al. (1998) Retinal A2E and mitochondrial dysfunction synergistically impair phagocytosis by retinal pigment epithelial cells. J Biol Chem 273: 24770–24778.
12. Jones MM, Manczur A, Schmitt C, Byrnes JC, et al. (2008) Mitochondrial DNA haplogroups and age-related macular degeneration. Arch Ophthalmol 126: 1235–1240.
13. Caniter JA, Olson LM, Spencer K, Schneitz-Boutaud N, Anderson B, et al. (2008) Mitochondrial DNA polymorphism A917G is independently associated with age-related macular degeneration. PLoS One 3: e2091.
14. Udari N, Aikano SK, Mentor GR, Menazrakhed M, Boyer DS, Clemons TE, et al. (2009) Mitochondrial DNA haplogroups associated with age-related macular degeneration. Invest Ophthalmol Vis Sci 50: 2966–2974.
15. SunGiovanni JP, Arking DE, Iyengar SK, Elshoff M, Clemons TE, et al. (2009) Mitochondrial DNA variants of respiratory complex I that uniquely characterize haplogroup T2 are associated with increased risk of age-related macular degeneration. PLoS One 4: e5508.
16. Pereira L, Goncalves J, Goios A, Rocha T, Amorim A (2005) Human mtDNA haplogroups and reduced male fertility: real association or hidden population substructuring. Int J Androl 28: 241–247.
17. Wiesbauer M, Meierhofer D, Mayr JA, Sperl W, Paulweber B, et al. (2006) Multiplex primer extension analysis for rapid detection of major European mitochondrial haplogroups. Electrophoresis 27: 3864–3868.
18. Koller B, Mueller EE, Eder W, Stanger O, Maier R, et al. (2009) Mitochondrial DNA haplogroup T is associated with coronary artery disease and diabetic retinopathy: a case control study. BMC Med Genet 10: 55.
19. Mueller EE, Eder W, Ebner S, Snaeager E, Santic D, et al. (2011) The mitochondrial T16189C polymorphism is associated with coronary artery disease in Middle European Populations. PLoS One 6: e16455.
20. Ebner S, Lang R, Mueller EE, Eder W, Oedler M, et al. (2009) Mitochondrial Haplogroups, Control Region Polymorphisms and Malignant Melanoma: a Study in Middle European Caucasians PLoS One. In press.
21. van Omen M, Kayser M (2009) Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 30: E86-E894; http://www.phylotree.org.
22. Brandtstatter A, Zimmermann B, Wagner J, Golub T, Rock AW, et al. (2008) Timing and deciphering mitochondrial DNA macro-haplogroup R0 variability in Central Europe and Middle East. BMC Evol Biol 8: 191.
23. Imamura Y, Noda S, Hashizume K, Shinoda K, Yamaguchi M, et al. (2006) Drusen, choroidal neovascularization, and retinal pigment epithelial dysfunction in SOD1-deficient mice: a model of age-related macular degeneration. Proc Natl Acad Sci U S A 103: 11202–11207.
24. Yacoubi Lousslati B, Tousti W, Cherni I, Rhomdhanie KB, Mota-Vieira L (2010) Germline HVR-II mitochondrial polymorphisms associated with breast cancer in Tunisian women. Genet Mol Res 9: 1690–1700.
25. Schmuczerova J, Behlke R, Doual M, Team RJ, Topinka J (2009) Genetic variability of HVRII mtDNA in cord blood and respiratory morbidity in children. Mutat Res 666: 1–7.
26. Meyer S, Weiss G, von Haeseler A (1999) Pattern of nucleotide substitution and rate heterogeneity in the hypervariable regions I and II of human mtDNA. Genetics 152: 1103–1110.
27. Mueller EE, Eder W, Mayr JA, Paulweber B, Sperl W, et al. (2009) Mitochondrial haplogroups and control region polymorphisms are not associated with prostate cancer in Middle European Caucasians. PLoS One 4: e6370.

PLOS ONE | www.plosone.org 6 February 2012 | Volume 7 | Issue 2 | e30874

MtDNA Haplogroups Associated with AMD

Drusen, atrophy or pigment epithelium changes). Patients with secondary CNV resulting from myopia, inflammatory or infectious chorioretinitis, angioid streaks, hereditary diseases or trauma were not included in the present study.

In all participants a complete ophthalmological examination, including dilated fundus examination, was performed. Distance visual acuity was measured at a distance of 4 m using Snellen charts. When CNV was suspected, additionally fundus photography, fluorescein angiography and optical coherence tomography (OCT) were conducted. Fundus photography and fluorescence angiograms were obtained digitally with a Zeiss fundus camera and imaging software (FODAS), and central macular thickness was measured with a Zeiss-Humphrey OCT Stratus 3000 (Jena, Germany).

2. de Jong PT (2006) Age-related macular degeneration. N Engl J Med 355: 1474–1485.