Reviewer's report

Title: Gender Bias Association with the Mitochondrial Haplogroup JTU Cluster in Patients with Age-related Macular Degeneration: A Case Control Study

Version: 1 Date: 25 July 2012

Reviewer: Barbara Kofler

Reviewer's report:

In their manuscript “Gender Bias Association with the Mitochondrial Haplogroup JTU Cluster in Patients with Age-related Macular Degeneration: A Case Control Study”, Kenney et al. analyzed the mitochondrial haplogroup cluster JTU and haplogroup H as well as the nuclear susceptibility polymorphisms ARMS2-rs10490924 and CFH-rs1061170 in 174 patients with AMD and 182 controls.

They report that the mitochondrial haplogroup cluster JTU, and the risk alleles of ARMS2-rs10490924 and CFH-rs1061170 are significantly associated with AMD, independently of each other and without an additive risk. Moreover, the association of the haplogroup cluster JTU is greater in males than in females.

The result is interesting and relevant for publishing, as in epidemiological studies it is important to obtain repetitive results in different studies.

Major Compulsory Revisions:

1) The reviewer is concerned about the use of samples in this manuscript and in previously published manuscripts (Udar et al. 2009, Mitochondrial DNA Haplogroups Associated with Age-Related Macular Degeneration; Kenney et al. 2010, Characterization of Retinal and Blood Mitochondrial DNA from Age-Related Macular Degeneration in Patients).

The manuscript of 2009 already documents higher frequencies of control region SNPs commonly found in haplogroups J, T and U in blood DNA of AMD patients compared to controls.

Are these blood DNAs as well as the work done for the previous manuscripts partly overlapping the data of this manuscript?

Although the manuscript of 2010 does not report haplogroup specific polymorphisms, the data in this manuscript (sequencing of the entire mtDNA) would be overlapping to the present manuscript if the DNAs of the individuals were partly overlapping.

It seems that all studies were done under the same ethics approval.

2) Although the reviewer assumes that only Caucasians were included in the study the information on the criteria for the inclusion have to be given in the methods section. Furthermore, were the age-matched controls recruited by the same study center and did they also have an ophthalmic examination to exclude presence of AMD. For what reason did the controls visit the hospital? Please also
indicate the geographic region from which the patients and controls were recruited. This information is very important to know because mitochondrial haplogroup distributions can vary dramatically between geographic regions.

3) Although the authors state that the case and control groups are age-matched, there is still a mean age of 74.88 in the controls and a mean age of 79.67 in patients with AMD. The information on the median age has to be added to table 1.

Is this age difference and also the difference in sex distribution statistically significant between patients with AMD and controls?

Age and sex correction (for the female and male results only age correction) should be performed.

In the Conclusion section, the sentence “This association was greater in males (OR=3.86, p=0.005) than the female populations (OR=2.37, p=0.013).” should be taken with care because the number of cases in each sex group is fairly low. This fact should be mentioned as a limitation of the study.

Minor Essential Revisions

1) Paragraph two of the background section: no space before the citation

2) In the fourth paragraph of the background section the authors mention of studies regarding Alzheimer’s and Parkinson’s but citation 14 is a paper regarding MS.

3) In the Methods section (DNA amplification) the forward primer given for the ARMS2 polymorphism is unclear because there is an A behind the 3’.

4) In the Methods section (Statistical analysis) the Fisher’s exact test is spelled wrongly with a c.

5) In the Results section, paragraph 1, the sentence “These findings show that AMD is more commonly found in subjects with the JTU mitochondrial haplogroup cluster than the H haplogroup” should be: “These findings show that AMD is more commonly found in subjects with the JTU mitochondrial haplogroup cluster than in subjects with the H haplogroup”.

6) In the Discussion section (paragraph four) a “that” is missing in the sentence: “It is possible that nuclear modifier elements could influence this gender bias.”

7) In the Discussion section (paragraph five) a full stop is missing after the sentence “Shortly thereafter, Schmidt et al., conducted a study that also identified the G>T polymorphism in ARMS2-rs10490924 as an AMD-susceptibility allele [58].”

8) Are there legends for the Tables? Abbreviations are neither explained (e.g. NL in table 2) in the Tables, nor in the text of the Methods or the Results sections of the manuscript.

Discretionary Revisions

1) In the Methods section (Mitochondrial haplogroup analyses) the H defining SNPs are indicated to be C7028T and A73G, but correctly it should be T7028C
and G73A.

2) The sentence “From these case-control studies, possession of the T allele for gene ARMS2-rs10490924 significantly increases one’s susceptibility to acquiring the disease but this risk is independent of the haplogroup background”, in the second last paragraph of the Discussion section, is not complete.

3) In the Figure Legend of Figure 2, it should probably mean GT instead of ST??, in the last sentence.

4) Table 4A: Haplogroups and Genotypes in AMD and Normal Populations: Why is there used a plural for populations?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests