Reviewer's report

Title: Mitochondrial DNA haplogroup T is associated with coronary artery disease and diabetic retinopathy: a case control study

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Reviewer: Tomomi Fujisawa

Reviewer's report:

Comments to the Author

In this paper, mitochondrial haplogroups were investigated in a Caucasian population of central- and southern Austria, focusing on coronary heart disease and diabetic retinopathy. The peripheral leucocytes were subjected to PCR-based typing of mtDNA haplogroup and the authors found a significant association of one mtDNA haplogroup with angiographically documented CAD, as well as with diabetic retinopathy in diabetic subjects. Several issues are concerned, and the followings are suggested:

Major comments

#1. The reviewer is concerned about statistical issue, especially multiple comparison. Since the mtDNA haplogroups consist of more than 5 groups, “significant” results are more likely to be pseudo-positive. It is not clear to the reviewer how such multiple comparisons would have affected the results, and how the multiple comparison issue has been taken into account in other published genetic studies on mtDNA haplogroup.

#2 The possible population stratification is of another concern. Since these two areas (Salzburg and Graz) were distant, it is possible the genetic background of the two groups would differ. As a result, I am afraid the main results of the present study could be biased. Can the authors address this concern by adopting proper testing? How about the difference in the distribution of mtDNA haplotypes between CAD patients in Salzburg and those in Graz?

#3 As the study population consists of three independent surveys and the distribution of mtDNA haplogroups were simply compared, especially compared with categorical subdivided groups. The authors would discuss on possible involvement of unknown cofounding factors in the result. Is it necessary to adopt an adequate statistical method to avoid pseudo-positive results in such a strategy?

Minor comments

#4 Introduction can be shortened

#5 Regarding a methodological issue, was the PCR-based typing for mtDNA haplogroup performed in a single center? If not, the authors may discuss whether typing in different institutions would affect the result.
The authors may show the frequency of T haplogroup of those with both CAD and retinopathy, in an available data set (at least 15% of retinopathy group had both).

Regarding heteroplasmy of mitochondrial DNA, it is unclear to the reviewer how the typing result would be affected under heteroplasmy. Is it possible that individuals that were not typed would have a high degree of heteroplasmy? In addition, some comments would be suggested for possible diversity in distribution of mitochondrial DNAs between peripheral leucocytes and vessel walls in an individual.

According to the description, 0.2% and 1% of samples were not included, because of the methodological failure. The total number of subjects investigated would be required.

The “coronary heart disease (n=1585)” for the CAD association study are unclear in the present form. As well, it is unclear to the author how the “heart disease other than coronary heart disease n=1296” were defined.

“Diabetic(s)” should not be used as a noun.

The reviewer is interested in a trend chi-square test for assessing association of the haplotype T with severity of retinopathy; i.e. no, non-proliferative and proliferative retinopathy.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

'I declare that I have no competing interests'