Reviewer’s report

**Title:** Lack of association between polymorphisms of the IL18R1 and IL18RAP genes and cardiovascular risk: the MORGAM Project

**Version:** 2  **Date:** 1 December 2008

**Reviewer:** Andrew J Sandford

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Synopsis:

The objective of this paper is to investigate a potential association of polymorphisms in the IL18 receptor genes: IL18R1 and IL18RAP with susceptibility to developing CVD. Moreover, as one polymorphism in the IL18 gene was previously associated with risk of CVD, this group was interested in studying potential epistasis between SNPs in IL18 and IL18 receptor genes.

No association was found between single SNPs or haplotypes in the IL18 receptor genes and risk of CVD. No interaction was found between SNPs in the IL18 receptor genes and IL18 gene SNPs and smoking status.

**Major Compulsory Revisions**

1. In the background section, the rationale provided for studying the IL18 receptor genes entirely relates to IL18. It would be informative for the authors to elaborate on their gene selection, for example, were the IL18R1 and IL18RAP genes picked up in genome-wide scans? Is there any other direct evidence of their plausibility as candidates for susceptibility to CVD?

2. Results section:

2.1. In the first paragraph of the results section, the abbreviation CHD is used for the first time without stating its meaning, whereas all other abbreviations in the paper were explained.

2.2. In the IL18R1 gene results, the first sentence states there were “slight deviations from Hardy-Weinberg equilibrium” but contradicts this by stating “all genotypic distributions were compatible with HWE”. In the IL18RAP results, it is stated that all genotypic distributions were in HWE, but this is incorrect from the data presented in Table 2.

For the IL18R1 data, out of the eleven SNPs genotyped, three different SNP genotyping data show departure from HWE, with p values below 0.05. The authors should present the actual p values so that the reader can judge whether the deviations are “slight”.

Concerning the IL18RAP data, four departures from HWE were observed. Giving rise to more concern, is the fact that all of them are in the same cohort (PRIME/France). I believe that is in need of an explanation. It could be due to genotyping or plating errors or perhaps some contamination issues, or population
stratification in this particular cohort. In any case, this should be discussed and details of quality control in the genotyping process should be clearly stated (see below).

2.3. In the results for both IL18R1 and IL18RAP, it is not mentioned if correction for multiple comparisons was performed in order to obtain the p values discussed in this section.

In the case of IL18R1 +404SNP in the Swedish cohort, if the p value is corrected then it should not be considered borderline. If there was no correction performed then the authors’ conclusion that there is no association is reasonable. The same reasoning applies to IL18RAP +55/in7SNP in the FINRISK cohort.

3. Methods section:

3.1. Using only the first stroke and/or CHD event for patients with multiple events does not fully utilize the data. The presence of multiple events samples could be used as an opportunity to stratify the cases into subgroups, which could then be compared by genotype. While there is no evidence of association when comparing cases with controls, it is possible that those SNPs or a portion of them will only be relevant in cases and would therefore be indicative of severity of CVD.

3.2. What are the sex specific markers used as part of the quality control?

3.3. In the quality control section, the part about the exclusion of some samples for contamination is not very clear. If contamination occurred in the process of plating or genotyping, the data from the samples in the same plate (or genotyping format used) would all need to be discarded and genotyping or plating repeated until evidence of contamination is no longer present. To that effect, the genotyping plates should contain appropriately placed negative controls. It is important to clarify the process of quality control.

3.4. It is mentioned that 0.85% of blinded duplicates was discrepant, but no such information is given for the known duplicates (~2% of genotyped samples).

3.5. Which database was used to select the Tag SNPs and what were the selection criteria?

3.6. The justification to perform haplotype analyses is the linkage disequilibrium between SNPs of the same gene. But the SNPs used are Tag SNPs which by definition should be in very low LD. So was LD calculated in each cohort? It would be relevant to show these data.

3.7. In the statistical analysis section, it is mentioned that Mantel-Haenszel statistics were used to adjust for the cohorts’ heterogeneity. It would be worthwhile to briefly explain this approach for the sake of the non-statistician reader.

The five cohorts studied are quite different: three out of five are comprised of men only; two out of five cohorts’ samples were treated in a different setting; differences in polymorphisms’ frequency were observed between the cohorts.

3.8. It is important to include power calculations in a negative study such as this. It would strengthen the article while helping other researchers make more
informed decisions about pursuing the same line of work.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests