Reviewer's report

Title: Endothelial nitric oxide synthase gene polymorphisms and risk of diabetic nephropathy: a systematic review and meta-analysis

Version: 2 Date: 7 August 2013

Reviewer: Tomomi FUJISAWA

Reviewer's report:

As a third reviewer, I would like to discuss, first of all, a possible explanation for the diverse opinions between the authors and one of the reviewers, and then propose ways to solve the issue(s).

Regarding the strategy to select controls in a genetic study for a predisposition to diabetic complication, I agree with the authors. Academically, however, the comments raised by the reviewer are somewhat suggestive. When the controls are defined as non-diabetic subjects as proposed by the reviewer, the observed association could reflect a genetic predisposition for individuals to develop “diabetic nephropathy”. The obtained results could reflect a mixture of a susceptibility to diabetes per se and that to nephropathy (after the development of diabetes), which cannot be discriminated. In this regard, to serve non-diabetic individuals as controls seem rationale to estimate a risk of “diabetic nephropathy.” However, from clinical points of view, most of medical staff would be interested in risks for (diabetic) nephropathy among individuals with diabetes, as in the case with hyperglycemia, rather than combined risks for developing diabetes and for nephropathy thereafter. That’s why diabetic individuals showing no or little nephropathy despite a term of duration have been widely investigated as controls, in most of the previous studies. It is likely, thus, that the opposite comments between the authors and the reviewer are derived from the different standing points; a clinical aspect and a bio-mathematic research.

The followings are suggested;

#1. The authors may wish to change expressions as for “nephropathy” risk, especially with caution to dissociate risk for diabetes and that for nephropathy, to avoid confusion of the readers of BMC genetics.

#2. How about adding the context above in the discussion?

#3. For more adequate interpretation of the present findings, it may be helpful to compare the ORs between the previous meta-analysis and the present one. If the distinct strategies in choosing controls are properly discussed, the comparison itself would give us more appropriate aspects in the possible role, if any, of the locus in the genetic predisposition to nephropathy. The authors are strongly encouraged to discuss these points academically in a more balanced manner.

#3-a. In this discussion, the authors may refer an updated published meta-analysis of the polymorphisms with type 2 diabetes, and discuss with the
present findings.
#3-b. Moreover, to see the difference between the two meta-analyses, number of studies and/or subjects that met the criteria to perform meta-analysis would be given.

Besides these points, I am concerned about;
#4. Although the authors described that “publication bias was observed”, it is not clear in the present form how did they reach the conclusion with the possible bias taken into account. This is a critical point for this meta-analysis. The authors should clearly demonstrate how the putative true effect was dissociated from the effect of the possible bias. It is reasonably suggested to show funnel plots for a dominant or recessive model, rather than allele contrast model, in a standard fashion. I agree the reviewer on this point (in the comment #5 in the 2nd report).

#5. In the discussion of the strength of this locus to the susceptibility, the descriptions of “minor” may well be reconsidered, provided that no strong genetic loci have been identified so far. The authors are also encouraged to show rationale for the population attributable risk.

#6. It is not clear in the present form whether the reported polymorphisms are in linkage-disequilibrium.

#7. Given the high impact factor of BMC genetics, this article may well be read by clinicians and medical staff who are not familiar with the difference between co-dominant and additive models, particularly the meaning of OR in each model. The authors are requested to add some explanations on these points.

#8. Diabetic(s) should not be used as a noun.

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

**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'