Reviewer’s report

Title: N-acetyltransferase 8, a positional candidate for blood pressure and renal regulation: resequencing, association and in silico study

Version: 1 Date: 20 November 2007

Reviewer: James Pankow

Reviewer’s report:

General

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Data collection methods for the association studies are described in insufficient detail to evaluate the validity of the design and results. An unpublished report by Org et al. on the HYPEST consortium is referenced, so the reader does not have access to supplemental information. For the study of long-term blood donors, relevant questions include: (a) participation rate; (b) methods for blood pressure measurement, including equipment used, whether there was a standardized, written protocol, and whether technicians were centrally trained and regularly monitored; (c) methods for ascertainment of cardiovascular disease and diabetes and their definitions; (d) methods to determine use of antihypertensive medication. For the essential hypertension cohort, relevant questions include: (a) participation rate; (b) methods to ascertain essential hypertension and its definition; (c) methods to ascertain hypertensive nephropathy complications and their definition.

2. Linkage disequilibrium patterns between SNPs should be characterized, as this information would be helpful in interpretation, particularly consistency of results across SNPs.

3. The study populations available for association analyses are relatively small and p-values are not impressive, given the fact that at least 18 separate tests were conducted in table 4. Replication of results for SBP and eGFR in other study populations would help reduce concerns that findings are simply due to type 1 error.

4. It is unclear why the authors performed parallel association tests with serum biomarkers for lipid metabolism or what is meant by “spurious associations due to inner structure of the study.” It is possible that the authors were concerned about confounding due to hidden population stratification, which would depend on allele frequencies and phenotype distributions across subpopulations. Finding no association between SNPs and other phenotypes (lipid traits) does not exclude the possibility that confounding exists for the traits of interest (SBP, DBP,
eGFR).

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

5. If available, laboratory quality control indices should be provided for biomarkers used in eGFR calculations (urea, SUN, creatinine, albumin), as the validity of eGFR depends on high quality measures for these biomarkers.

6. The first sentence after the methods/results heading in the abstract includes the phrase “kidney metabolism regulation”. This should be changed to “kidney function”, as the study is of an estimate of kidney function rather than regulation of kidney metabolism.

7. Page 7 of the manuscript includes the phrase “hypertensive neuropathy”, this should be changed to “hypertensive nephropathy”.

8. Page 9 contains a description of the alignment of NAT8 protein sequences with other “mammalian” species. However, Xenopus tropicalis is not mammalian. This should be reconciled.

9. Page 11 contains results of 157 subjects used in the kidney function association analysis. This sample size differs from that initially outlined in the methods (n=161), suggesting that 4 participants were excluded in the analysis. The authors should comment on why this was the case.

10. In table 4, reporting all p-values, regardless of significance, is more informative than substituting “ns” for p-values that exceed 0.1.

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Discretionary Revisions (which the author can choose to ignore)

11. A table summarizing basic characteristics (age, BMI, etc) of the populations used (42 subjects for resequencing, 137 subjects for BP association, 161 subjects for kidney function association) would be useful. In addition, reporting the severity or duration of hypertension, treatment if any, and presence of diabetes in the hypertensive cohort would be of interest.

12. Evaluating the association between NAT8 variation and the presence or absence of hypertensive nephropathy would be of interest to readers in addition to renal function reflected by estimated GFR.

13. The authors should consider expanding the discussion to include the study’s limitations. For example, discussing the potential for bias due to phenotypic misclassification/heterogeneity or discussing the potential for type I error due to multiple comparisons and small sample size. Acknowledging the limitations, especially the lack of sensitivity, of using a serum creatinine-based GFR estimating equation would also be helpful.
14. Discussing any connections between the kidney’s response to nephrotoxins, cited as a possible explanation for the protective role of NAT8 variants, and the pathogenesis of essential hypertension or hypertensive kidney disease would be of interest. Is there presently any evidence for overlap between these distinct disease processes?

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

**Quality of written English:** Needs some language corrections before being published

**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.