**Author's response to reviews**

**Title:** Suggestive linkage detected for blood pressure related traits on 2q and 22q in the population on the Samoan islands

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**Author's response to reviews:** see over
Dear Editor in Chief,

Please find enclosed our revised manuscript entitled “Suggestive linkage detected for blood pressure related traits on 2q and 22q in the population on the Samoan islands” by Karolina Aberg et al. and supplementary material.

We organize our response by replying to each reviewer’s comments after each of their comments. All authors have seen and approved this revised manuscript for submission. The authors have no conflict of interest to declare. We look forward to a favorable editorial decision about our revision. Thank you for your consideration.

I will be the corresponding author as before.

For the authors,

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Response to reviewers:
We appreciate the close review of our manuscript and the helpful comments. We reply to each comment underneath.

To reviewer Norihiro Kato,

Minor Essential Revisions:
1. The relevant information may have been described in the previous reports but it would be helpful to show the relationship between blood pressure (and hypertension prevalence) and participants’ age, i.e., how ageing influences the blood pressure increase or prevalence of hypertension, in the tested population.

Response: We have followed the reviewers advice and have added the Pearson’s correlation between the phenotypes and age and the prevalence of hypertension for two age groups (<40 and ≥ 40 years old) to Table 1.

2. Since the sample size per pedigree spans from 2 to 246, it may be possible to evaluate whether predisposition to hypertension is rather prominent in some (large) pedigrees with more affected individuals. The reviewer would recommend the authors to perform the test of linkage among such large families, which may enhance the power of linkage to some peaks and may increase the LOD scores
accordingly.

**Response:** The referee recommend performing linkage investigations within subsets of our study sample, where each subset would consist of a single large family, in order to potentially increase the LOD scores for some peaks. While we agree with the reviewer that such studies of subdivisions of the sample might be interesting, such analyses are more appropriate for a simple Mendelian disease than for a complex trait like hypertension. Subdividing the samples to single-family based units increases the risk of detecting false positive signals and signals that potentially are interesting for only the investigated family. It would also substantially lower the power to detect linkage, by decreasing the effective sample size to that of one family. Based on the assumption that the environmental factors in the two polities are somewhat different, we have currently subdivided the study sample into two subpopulations based on residential origin. In order to identify loci of major effect for the general population with high power while minimizing the risk of detecting false positive signals, we feel that performing additional low-powered testing in single-family subdivisions of the sample is not appropriate for a complex disease like hypertension.

Discretionary Revisions

1. Because hypertension is a late-onset disease, it would be helpful to know the results for testing linkage of hypertension when the participants are restricted to middle-aged and older people, e.g., >40 years.

**Response:** As the reviewer points out, older age is an important risk factor for the development of high blood pressure. In order to account for this risk factor, we have performed covariance screening, including four factors involving age (age, age*sex, age^2 and age^2*sex), prior to conducting variance component linkage analysis. As shown in Table 2, one or several age-related factors are of significant importance for each of the investigated traits and are therefore included as covariates in the linkage analysis. Thus, we have already taken age-effects into account while testing for linkage. As mentioned above, in order to identify loci of major effect for the general population with high power we do not wish to subdivide our study sample further.

2. The heritability seems to be modest as compared with the figures reported in other populations. Please describe whether this is specific to blood pressure phenotypes or also applicable for other metabolic traits such as lipid in the tested population.

**Response:** We have followed the referee’s advice and have now added a new section in the Discussion under the heading “Heritability” about heritability of blood pressure in Pacific study populations. The heritability of the blood pressure traits are somewhat lower than those we estimated for lipid and lipoprotein traits in a recent report (Aberg et al. 2008. A genome-wide linkage scan identifies multiple chromosomal regions influencing serum lipid levels in the population on the Samoan islands. *J Lipid Res* 49(10):2169-2178). Comparing heritability across different phenotypes is difficult due to differences in sets of covariates, and technical measurement errors. We are not convinced it is important to make such comparisons for the purposes of identifying potential QTLs. Our purpose in estimating and reporting the heritabilities was to establish that there was
genetic variation in the BP traits and that we had an opportunity to detect genomic regions associated with these phenotypes.

To reviewer Nora Fraceschini,

Major compulsory revisions:
1. The context for studying blood pressure and hypertension in Samoan islands individuals should be given, i.e., prevalence and incidence of hypertension in this population, impact of hypertension in morbidity and mortality and so on. The heritability of blood pressure traits is low for a described homogenous (? isolated) population. Please comment. If available, compare these findings to populations of similar ancestry.
   **Response:** We appreciate the reviewer’s suggestion and have added more descriptive results about levels of hypertension in the study sample (see our reply to item #1 of Dr Kato). We also added a paragraph in the Background on prior blood pressure studies in Samoans. We discuss concisely the heritability levels and compare to them prior Samoan studies and other Pacific populations.

2. The discussion should also include genetic findings from candidate and genome wide association studies of genes located under the linkage peaks.
   **Response:** We would like to strongly emphasize that although the current linkage study has the potential to identify susceptibility loci for the investigated traits, further investigations of these loci are necessary in order to identify any specific genes involved. We have added the following text to the discussion:
   *Despite the detection of two susceptibility loci on chromosome 2q and 22q in multiple studies, to our knowledge, no verified candidate genes for blood pressure related traits have yet been identified in these regions.*

   *Recently a number of genome-wide association studies [40-47] on blood pressure related phenotypes were reported. While these studies found significant associations on multiple chromosomal regions none of them reported any major findings on chromosome 22. However, one study [42] reported significant associations with single nucleotide polymorphisms (SNPs) on chromosome 2q. Although these sequence variants are located on the same chromosomal arm as some of our findings, the closest significant marker is located approximately 25 Mbp centromeric to our linkage signal, and thus there is no direct overlap between our major linkage results and current GWAS.*

3. Power to detect an effect may be a concern in this analysis. Please include the number of relative pairs used for the analyses.
   **Response:** Detailed tables of the relative-pairs have been published previously. In order to briefly describe the study samples we have added the following sentence to the method section: *As described in detail previously [3, 4] the American Samoan and the Samoan study-samples consist of 1,465 and 1,633 genotyped and phenotyped relative-pairs, respectively, useful for linkage studies.*
4. Please define the covariates: smoking, alcohol intake and physical activity. Is physical activity self-reported?

Response: We have reworded the description of the covariates to clarify that the information was self-reported. The updated sentence reads: *Questionnaires were used to collect self-reported information on environmental factors including education (years), moderate to heavy physical activity (hours/week), alcohol consumption (yes/no), smoking (yes/no) and material life standard.*

Minor Essential Revisions:
1. Table 1, add relative pairs

Response: We have added a sentence describing this in brief and have added references to where careful details previously have been published. (See text above.)

2. Table 1; are the mean blood pressure levels already adjusted for the medication use?

Response: The mean measure of blood pressure in Table 1 was not adjusted for medication. We have clarified this in the legend of Table 1 by adding the following sentence: *Calculations are made prior to correction for medication.*

To reviewer Tetsuro Miki

1. Some genome-wide association studies for hypertension have reported recently, authors should refer to these studies and compare the responsible regions between the Samoan populations and other populations, Caucasian and Asian populations.
   (4) Yoon Shin Cho1, et al., A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet. 2009 April 28. [Epub ahead of print]

Response: The GWAS listed by the reviewer were reported while our manuscript was under review and we were therefore not able to include these studies when initially writing the manuscript. However, we have now added a brief paragraph discussing the overlap between these GWAS studies and our linkage investigation. See response two to Dr Fraceschini.

2. Please describe the candidate genes for the blood pressure traits on two chromosomes, 2q35-37 and 22q13.

Response: See response to Dr Fraceschini’s comment above.
3. Authors published two regions for adiposity-related phenotypes and lipid-related phenotypes in the Samoan samples, please describe about the common chromosome regions which may be responsible for Metabolic Syndrome, such as obesity, dyslipidemia and hypertension.

Response: We have added the following paragraph to the end of the discussion:

The overlap in genomic regions identified between our previous studies on adiposity- and lipid-related phenotypes and our current study is negligible [2-6]. Taken together these results may suggest that no common chromosomal regions with major effects for the metabolic syndrome exist within the studied population. However, considering the different levels of heritability detected for the different phenotypes in the study samples, our statistical power to detect shared genetic effects may be limited. Future work remains to explicitly test for pleiotropy across metabolic, lipid and cardiovascular phenotypes in the Samoan study sample.