Author's response to reviews

Title: Differences in serum SP-D levels between German and Japanese subjects are associated with SFTPD gene polymorphisms

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Author's response to reviews: see over
Differences in serum SP-D levels between German and Japanese subjects are associated with \textit{SFTP D} gene polymorphisms

Dear Dr Giorgio Sirugo

Thank you very much for your reply. We would like to submit the revised manuscript that addresses the comments from the reviewers. We revised our manuscript using 'track changes' of MS Word. In addition, please find a detailed account of how each of the concerns of the reviewers has been addressed.

We greatly appreciate the opportunity to resubmit our work to \textit{BMC Medical Genetics} and hope that it now meets approval for publication.

Sincerely, Yours,

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Replies to the reviewers’ comments:

**The Reviewer 1: Dr. Branwen Hennig**

Major Revisions

1) Why was genetic variation screened only in the SFTPD but not the SFTPA1 or SFTPA2 genes? Please explain briefly.

Response:

Because ethnic difference between German and Japanese was found only in serum SP-D levels but not in SP-A levels, we investigated SNPs only in the SFTPD but not in the SFTPA1 or SFTPA2 genes. This point is clarified in the revised manuscript (page 9).

2) How were the four SNPs for genotyping in the SFTPD gene selected? The authors state that the genotype distributions are different in HapMap CEU and JPT, but they do not describe if they used a cut-off for selection by genotype frequency difference. Are these SNPs functionally relevant? Information is given on some, but not all of the four SNPs investigated?

Response:

We agree that the reviewer’s concern is reasonable. The SNPs in the SFTPD gene investigated in this study were chosen as follows. First, we analyzed HapMap genotyping data to identify SNPs that show different genotype distributions between HapMap CEU and JPT by performing chi-square test. We then selected these four SNPs that have been reported to be associated with serum SP-D levels and/or TaqMan Fast PCR assays (Life Technologies Corp) are available for. This process to select the SNPs is also described in the revised manuscript (page 9).

3) The reporting of the genotype data would seem more logic if the authors had first described the within-population and then the between population comparisons with regard to both SP-D level and disease status.
Response:
In response to the reviewer’s comment, we revised Figure 3 to show within-population comparisons of serum SP-D levels according to the four SNPs. These results are also described, in the Results section of the revised manuscript (page 12 to 13).

*The multivariate analysis tested effects of individual SNPs on SP-D level adjusted for disease status, age and ethnicity. Maybe I misunderstood, but shouldn't the question be whether SP-D level (as predictor and diagnostic biomarker) affects disease status (as distal outcome), adjusted by SNPs, age and ethnicity?*

Response:
We agree that the reviewer’s comment is reasonable. However, the aim of multivariate analyses was to determine whether SNPs in *SFTD* independently affect serum SP-D levels even when adjusted by the other cofactors. It is beyond the focus of our study to test the correlation between “disease status” and serum SP-D levels. In the revised manuscript, we tried to emphasize the aim of the multivariate analyses (page 10, 13, and 16).

*Why are the results for the multivariate analysis for rs3088308 not shown in table 2?*

Response:
We performed multivariate analysis only when the SNP showed significant correlation with serum SP-D levels in a univariate analysis. Because the univariate analysis did not show significant correlation between rs3088308 and serum SP-D levels, the multivariate analysis for this SNP was not conducted.

**Minor Essential Revisions**

4) *Tables 1 and 2 would benefit from showing absolute numbers, % and totals for the different characteristics / outcome categories and genotypes in the two populations to facilitate the interpretation of the data by the reader.*
Response:
New Tables 1, 2 and 3 of the revised manuscript are constructed following the reviewer’s suggestion.

5) Table 2 should show the genotype distribution separately for cases and controls in both populations, for completeness.

Response:
We totally agree with the reviewer’s comment. The data shown in Table 2 of the previous manuscript are now split into new Table 2 showing the data of genotype distributions within each German and Japanese cohort and new Table 3 demonstrating the comparison data of genotype distributions between the German and Japanese cohorts in the revised manuscript. The description on these results is also added in the Results section of the revised manuscript (page 12).

6) Presumably HWE was calculated in the controls only? Please state this in the paper or table legend.

Response:
In new Table 2 of the revised manuscript, each HWE calculated for German patients with IIPs, German HS, Japanese patients with IIPs, and Japanese HS is listed.

Discretionary Revisions
7) Numbers <10 should be spelt out (e.g. page 9)

Response:
We spelled out numbers <10 in the revised manuscript.
The Reviewer 2: Dr. Digna R Velez Edwards

Major Revisions

1) The motivation for selecting these specific four SFTPD genetic variants is unclear. According to the methods they were selected because of their genotype frequency differences between HapMap CEU subjects and JPT, rather than because of any specific prior association with disease. The authors than go on to highlight the finding that there were genotype frequency differences between German and Japanese subjects, is this really an unexpected finding given the SNPs were selected for having different distributions across these two populations? Why didn’t the authors select SFTPD variants that had been previously associated and then examine for ancestral differences?

Response:

These specific four SNPs in the SFTPD gene were chosen as follows. First, we analyzed HapMap genotyping data to identify SNPs that show different genotype distributions between HapMap CEU and JPT by performing chi-square test. We then selected these four SNPs that have been previously reported to be associated with serum SP-D levels and/or the TaqMan Fast PCR assays (Life Technologies Corp) are available for. The process to select these four SNPs is detailed in the revised manuscript (page 9).

As the reviewer suggested, if there are SNPs in the SFTPD gene that had been associated with IIPs, it would be more interesting to investigate the difference in genotype distributions of such SNPs between the German and Japanese cohorts. Unfortunately, however, the presence of such SNPs has not been demonstrated. We thus selected these four SNPs as mentioned above because they might have associations with serum SP-D levels and ethnic differences in genotype distributions. In fact, there have been no previous reports that directly compared the ethnic difference in genotype distribution of these SNPs and/or proved the associations between these SNPs and serum SP-D levels. Therefore, we should say that the motivation that selected these four SNPs was to thoroughly investigate these ethnic differences and associations in our German and Japanese cohorts, and we hope that the reviewer understand these points.
2) The authors indicated that in their multivariate model they included ethnicity, do they mean they combined German and Japanese subjects or did they have another “ethnicity” covariate among German and among Japanese? … If they combined German and Japanese subjects then the analyses are inappropriate and they should perform analyses stratified by German and Japanese subjects, particularly given that we know there are genotype frequency differences between these two populations for these SNPs.

Response:
We agree that the reviewer’s concern is reasonable. In the multivariate model, we combined German and Japanese subjects because we intended to determine whether the SNPs in the SFTPD gene affect serum SP-D levels independently from other covariates including ethnicity and whether the impact of SNPs on serum SP-D levels was greater than that of ethnicity. We examined multicollinearity in our multivariate models by calculating variance inflation factor (VIF), and confirmed that there was no significant collinearity between the genotype of SNPs and ethnicity. We thus believe that our multivariate analyses were appropriate to be conducted. To clarify these points, VIF values are added in new Table 4 of the revised manuscript.

Minor Essential Revisions
3) Two of the SNPs (rs72917 and rs2243639) result in amino acid changes in the literature (missense) and one has previously associated with emphysema, interstitial pneumonia, and lung cancer (rs721917), an expanded discussion of the results in the context of this would be helpful.

Response:
We totally agree with the reviewer’s comment. We expanded discussion on these points in the revised manuscript (page 16).

4) The authors note in Table 1 that the healthy subjects differed for age and sex by the study populations (German and Japanese). Can the authors provide additional details
regarding control selection across the two populations, were there differences in the study protocol for controls across the two populations?

Response:
The differences in age and sex between German HS and Japanese HS were caused by the difference in health checkup system between Germany and Japan. We briefly explained this point in the revised manuscript (page 8 and 16).
The Reviewer 3: Dr. Paola Borgiani

Minor Revision

In the Figures, the title (Fig 1, Fig 2..) it is not very well readable.

Response:

We corrected these points following the reviewer’s suggestion.