Reviewer's report

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

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Reviewer: Branwen Hennig

Reviewer's report:

The study by Horimasu and colleagues describes the investigation of serum levels of SP-D and SP-A as well as four polymorphisms in the SFTPD gene (encoding surfactant protein D) in the context of idiopathic interstitial pneumonias (IIPs) in Japanese and Germans.

This is an interesting albeit small study showing differences in both serum SP-D levels and genotype frequencies between the two populations, which is likely to have important implications in the application of SP-D as diagnostic biomarker in different ethnic groups.

However, a number of details are unclear in both the materials & methods and results sections, which should be addressed, to explain how the above conclusions were drawn.

• Major Compulsory Revisions

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

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 – these could be explored using bioinformatical tools where little information is available from the literature. How are the SNPs spread across the gene and do they tag one another or other SNPs in the gene, i.e. how informative are these four markers? Consequently it is also not clear whether a haplotype analysis might have been warranted.

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.

The results show that i) in both populations SP-D levels were higher in IIPs cases compared to controls, ii) SP-D levels were higher in both German IIPs cases and controls compare to Japanese cases and controls, iii) three out of the four SNPs tested were associated with SP-D level in both populations and iv) the genotype distribution did not differ by disease status in either the Japanese or Germans.

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? Such an analysis would presumably show that the association between SP-D level and disease status across populations is biased by population substructure, evident in the differences in genotype frequencies between Japanese and Germans.

Therefore, the presentation of SP-D level by disease status and genotype combined for both populations (as shown in figure 3) does not make sense to me. However, the conclusions drawn by the authors, namely variability in SP-D level is at least in part explained by SFTPD polymorphisms in the two populations studied, does make sense.

Finally, why are the results for the multivariate analysis for rs3088308 not shown in table 2?

- **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.
  5. In addition, Table 2 should show the genotype distribution separately for cases and controls in both populations, for completeness.
  6. Presumably HWE was calculated in the controls only? Please state this in the paper or table legend.

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

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests