Author's response to reviews

Title: Association of FcgammaRIIa R131H polymorphism with idiopathic pulmonary fibrosis severity and progression

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Author's response to reviews: see over
Dr. Christina Chap  
Assistant Scientific Editor  
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19th August 2010  

RE: 6671262764119600 - BMC Pulmonary Medicine  
“Association of FcyRIIa R131H polymorphism with idiopathic pulmonary fibrosis severity and progression.”

Dear Dr Chap,

We would like to thank you for the thorough review of our manuscript and for the helpful and constructive comments of the referees. In the revised manuscript, we have made the necessary changes, which we believe address the reviewers’ suggestions. A detailed point-by-point response is attached. We hope that these changes and improvements to the manuscript will now make it acceptable for publication in *BMC Pulmonary Medicine*.

Thank you very much.

Sincerely yours,

Dr Simon P. Hart  
Senior Lecturer in Respiratory Medicine
Reviewer 1 – Martha Perez-Rodriguez

Major

1. We agree with the reviewer that the patient control group may have been exposed to some factors that contributed to the disease outcome, and for this reason it would be better to keep them separate from the healthy control group. However, it is important to clarify that there were also individuals with acute (as well as chronic) lung diseases within the patient control group. It is therefore unlikely that these patients were exposed to different factors than those in the blood donor sub-group. In addition, we would suggest that the factors that might have contributed to the different pathologies of diseases observed in the patient control group would be likely to be different for each patient and possibly would not have affected the observed genotype frequencies. This suggestion is supported by similar genotype frequencies observed between the blood donor control and patient control sub-groups.

2. We have now included details of the study design in the Methods sections, as suggested. (p. 4, lines 10-17)

3. The patient control group comprised randomly selected patients with a number of different acute or chronic lung pathologies, ranging from asthma and seasonal influenza to pneumonia and lung cancer that were admitted to the Respiratory Unit of the Edinburgh Royal Infirmary (p. 5, lines 3-6). We feel that a detailed list of each separate pathology and the number of patients would not add significantly to the present manuscript. Furthermore, as we did not observe any difference in the distribution of the R131H genotypes between the control and IPF groups or between the control patient group and healthy blood donors, inclusion of details of all the different pathologies in the methods section seems unnecessary. In the current study design, it was important that in the control patients group (i) none of them had (or ever had) interstitial lung disease (ii) the age of this group to be similar to that of the IPF cohort (71.4 ±10.2 for patient control vs. 70 ±8.8 for IPF).

4. We agree with the reviewer that it is indeed interesting that the ancestral allele (H131) displayed lower frequency than the R131 allele both in the control and in the IPF groups. One possibility would be that since the H131 allele represent the sole human leukocyte Fc receptor allotype capable of binding to IgG2, it might have been subject to strong evolutionary forces, which shaped R131H genotype distribution in order to confer protection from a number of autoimmune and chronic inflammatory disorders that are associated with the R131H polymorphism. This assumption could be further strengthened by the fact that the frequency of the R131H genotypes varies greatly between different ethnic groups (especially between Caucasians and Asians), suggesting the presence of different evolutionary forces in different populations (e.g. Botto et al. Clin Exp Immunol 1996 104:264-8; Chen et al. Clin Exp Immunol 2006 144:10-6). It should be noted that the genotype frequencies observed in this study are in line with those in several previously published studies in British and Caucasian populations (e.g. Botto et al. Clin Exp Immunol 1996 104:264-8; Salmon et al. J Clin Invest. 1996 97:1348-54).
5. Although we agree with the reviewer that the reported associations on disease progression could be related to the degree of lung inflammation, lung biopsies were not performed in all IPF patients, but only to those, for which the clinical and radiological findings were insufficient to establish a definite diagnosis. In addition, the time point that surgical biopsies were performed varied greatly between different patients. For this reason, direct comparison of lung inflammation in biopsy samples from different patients might not be feasible, as it might represent different stages of the disease. The number of patients that have undergone surgical biopsy is therefore not representative of the total IPF cohort and too low to allow us to correlate FcγRIIa R131H genotypes with lung inflammation with enough statistical power.

6. As the reviewer notes, we had mistakenly not included details of the post hoc test we performed. In the revised manuscript, we have now added details of the post-hoc analysis, as suggested. (p. 6, lines 22-24).

7. We have now improved the discussion to relate the results in FcγRIIa R131H genotypes with pulmonary function (p 10, lines 10-13), as suggested and made some modifications in the discussion section overall to place our findings in the context of IPF pathology.

Minor

1. We have now expanded the abbreviated titles of ATS/ERS and BAL. (p. 4, lines 19-20, 22)

2. The sequence of FCGR2A, which was used as the basis to design the set of primers used in the genotyping assay, was obtained from online repositories (NCBI) and not from previously published studies. As suggested, in the revised manuscript, we have now added details about the human genome sequence assembly that was used for primer design (p. 5, lines 24-25). Also, the rs designation for the FcγRIIa R131H polymorphism (rs1801274) is included in the revised manuscript and appears in the Abstract and Introduction sections (p. 2, line 4; p.4, line 1)

3. We have now included details on the PCR buffer concentration and the expected size of the PCR products in the Methods section. (p. 6, line 4 & 8)

4. Direct sequencing was only used to test the validity and specificity of the PCR-based genotyping and not for the determination of FcγRIIa R131H genotypes. We have therefore selected patient and control samples for subsequent analysis by direct sequencing, so as to confirm the results already obtained from the PCR-based assay. The number of samples corresponded to about 10% of the total analysed samples (n=366), with a minimum of 10 for each tested genotype (RR, RH, HH). In all cases, the genotypes obtained from the sequencing-based method matched that obtained from the PCR-based one. This point is now clarified in the Methods (p. 6, line 8-9).

5. The RR genotype was used as the reference for the comparisons with the HH genotype in the lung function data at baseline and at 12 months, because our data suggest that lung function is dependent on the number of H alleles. In particular, as it is illustrated in Figure
1A-C and Table 2, baseline pulmonary function and more specifically, FEV1, FVC, and DLCO, seems to correlate with the number of H alleles in each patient. For example, more severe restriction and gas transfer was evident in HH homozygotes compared to RH heterozygotes, and accordingly, in RH heterozygotes compared to RR homozygotes. This observation suggests a dose-dependent effect for the H allele on pulmonary function and therefore in disease manifestation. Similarly, a dose response relationship has been previously demonstrated for the R131 allele and SLE (Karassa et al., 2002 Arthritis Rheum 46, 1563-71). We have therefore used the RR genotype (0 H alleles) as reference for comparison with the HH genotype (2 H alleles).

**Reviewer 2 – Martin Petrek**

Major compulsory revision:

1. We agree with the reviewer’s point relating to the size of our IPF cohort. We have now acknowledged the need to perform additional studies to confirm the observed association as suggested. (p. 11, lines 9-12)

2. We have performed statistical power analysis to determine the power of our sample size to detect differences between the IPF and control groups, and also the power for replication of the observed difference in the minor allele frequency between IPF subgroups (severe/non-severe; progressive/non-progressive). Statistical power values are included in the revised manuscript. (p. 6, lines 16-21; p. 7, lines 1-2)

3. We have now added the appropriate nomenclature for SNP designation in the abstract and introduction sections. (p. 2, line 4; p.4, line 1)

Minor essential revision:

1. As suggested, we have now included the number of subjects (n) in the 2 control subsets (healthy donors and control patients) and demographic (age, gender) data (p. 5, line 3, 6-7). We are not able to include the precise age of healthy blood donors due to missing data.

Discretionary revisions:

1. FcγRIIa R131H genotypes were determined by allele-specific PCR as described in the Methods section. In order to validate the results obtained from the PCR-based assay, selected samples were analysed by direct sequencing or re-analysed using the PCR-based method. In all cases, there was complete concordance of results relating to genotype determination. We have therefore not included any details of the success/failure rate in the revised manuscript.
2. We agree with the reviewer about the presence of published studies that reported no association between CR1 gene polymorphisms and IPF (Kubistova et al. Tissue Antigens, 2008, 72:483-6; Hodgson et al. Respir Med 2005, 99:265-7). As suggested, we have now cited these reports in the discussion section. (p. 10, lines 19-20)