Reviewer's report

Title: Associations of ECP (eosinophil cationic protein)-gene polymorphisms to allergy, asthma, smoke habits and lung function in two Estonian and Swedish sub cohorts of the ECRHS II study

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Reviewer: Andrew Sandford

Reviewer's report:

Summary:
This article describes the associations of three ECP gene polymorphisms with allergy, smoking phenotypes, asthma and lung function (FEV1). Asthma and smoking phenotypes were taken from questionnaire data of the ECRHS II study; allergy and lung function were assessed in an ECRHS II study sub-cohort comprising Estonians and Swedish subjects.

The experimental design is sound and appropriate controls have been included. Information about the SNPs (and their rs numbers) as well as their assay information is provided.

The two racial components of the subcohort showed differences in atopy, smoking, gender distributions as well as allele frequency of the ECP434 SNP.

Combined analysis of both populations resulted in association of ECP434 with current smoking, lung function in females and with non-allergic asthma in females as well as association of ECPc.-38 with atopy in males.

In the separate analysis of the Swedish population, ECP434 was associated with gender, lung function in females, current smoking and non-allergic asthma. ECPc.-38 was associated with gender and asthma; whereas ECP562 was associated with lung function in females.

In the separate analysis of the Estonian population, ECP434 was associated with smoking and ECP562 with gender.

Major Compulsory Revisions

In the paragraph ‘Separate evaluations of the Swedish cohort’:

- The first sentence of the second paragraph should read ‘As shown above for the combined analysis, atopy…’

- You analysed non-smoking Swedish individuals separately because of the smoking effect you observed. Why was a logistic regression not performed to adjust for the confounding variables such as smoking and gender?

- You mention no smoking men from Sweden carried the ECP434CC allele. It would be helpful to readers to remind them of the number of such individuals and mention the Hardy Weinberg calculations for the ECP434 genotyping.
In the discussion:

- You mention a previous study reporting ‘very close relationships between these two genotypes’. The presumably refers to strong linkage disequilibrium. The linkage disequilibrium ($r^2$) should be calculated between the polymorphisms in your populations.

- How far is the ECP gene from the chromosome 14 region associated with successful smoking cessation in the GWAS? The linkage disequilibrium between the relevant loci in this region can be determined from the HapMap (or similar) data and should be presented. It is not correct to state “Any linkage [disequilibrium] between the ECP genes and either of these candidate SNPs is unknown”.

- You should attempt an explanation as to the contradiction of your results with the previous reports of ECPc.-38 association with asthma and allergic phenotypes in the Norwegian-Dutch study.

- You conclude that “…the activity of ECP may be of the greatest importance for the development of irreversible damage to the lung in smokers and pulmonary disease’. That is an overstatement; your study does not provide strong evidence to support it.

What is the reason for combining the Estonian and Swedish populations in the analysis? Their demographics and ECP434 allele frequency are different.

You do not mention correction for multiple comparisons for your association results. Was it done? If yes, you should mention which method was used; if not, your significant findings will not survive correction and that should be made clear to the readers.

What are the novel findings of your study? There were previous reports of ECP434 and ECPc.-38 polymorphisms with asthma and allergic phenotypes. You do not report any major finding for ECP562, which is the least studied polymorphism.

It was not clear to me why smoking was used as an outcome variable. Are the authors hypothesizing that ECP is involved in the tendency to smoke or to quit smoking?

Overall, the article lacks focus because there are too many analyses. It would be better to limit the analyses to the asthma-related outcomes and include gender and smoking as confounding variables, as discussed above. The combined analysis is potentially flawed due to the genotypic and phenotypic differences between the populations and therefore should be deleted. The two populations could then be treated as independent study groups.

Minor Essential Revisions

In the paragraph ‘Relations of ECP genotypes to atopy and asthma’, there seems to be a contradiction in the last sentence when you assert that there was no SNP
association with asthma but an association of ECP434 with non-allergic asthma. It would be better to specify allergic asthma in the first half of the sentence.

The number of subjects is given as 767 in the first section of the Methods but in the DNA extraction it is stated that there were samples from 465 and 292 subjects. The authors should address this discrepancy.

Why were two TaqMan assays used to genotype the ECP562 polymorphism? Authors should include the commonly used name for ECP: RNASE3.

The reporting of the association results for the separate Swedish analysis would benefit from some clarification and re-organizing. Perhaps a table would be more useful.

The word “relations” should be replaced by “relationship” throughout the manuscript.

Non-significant findings should not be referred to as “a tendency” or “somewhat higher”.

Level of interest: An article of limited interest

Quality of written English: Acceptable

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