Author's response to reviews

Title: Evaluation of variants in the selectin genes in age-related macular degeneration.

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Author's response to reviews: see over
March 1, 2011,

Dear Editors,

We wish to thank the reviewers for their careful review and constructive comments. We have revised the manuscript as suggested by the reviewers. A point by point response is included below:

Reviewer 1

1. The question was raised about selectin expression across different donors. Although we did not exhaustively characterize donor-to-donor variation, we have performed additional experiments to address this and added this information to the results section.

2. The reviewer notes that we did not cite a relevant manuscript by Bojanowski et al.; we have now added this to the discussion section.

3. The reviewer requests that we include Hardy-Weinberg p values for the control SNP frequencies. We have now added this information to Table 1. One SNP (rs6693963) did not meet our criteria for Hardy-Weinberg equilibrium and was removed from our analysis.

4. Use of the word “ancestral”-we respectfully suggest that our use of this word is appropriate in its application to association studies that are searching for common variants that are passed down from a population’s founders.

Reviewer 2:

1. The reviewer asks for additional information about the donor eye immunohistochemistry experiments, including location of the sections and numbers of eyes. We have now performed the P selectin experiments on 9 AMD and 9 control donors. This has been added to the Methods and Results sections.

2. The reviewer asks about AMD eyes; please see point 1 above. The reviewer also asks how the minor allele frequencies in our controls compared to those of the general population. We have a HapMap minor allele frequency column to Table 1.
3. The reviewer suggests adding 3 references to the Discussion. Two of these (Bojanowski and Yeh) fit very well and have been added. The Klein et al. MS describes serum biomarkers, and this is conceptually difficult to fit into the paper.

Reviewer 3:

1. The reviewer requests additional information about the samples used for RT-PCR and immunohistochemistry. This has now been added to the methods.

2. We have added a note on use of DAPI in the materials and methods and comment on autofluorescence of the RPE in figures 1 and 2 legends.

The immunolabeling of microglia (arrows in Figure 1) may have been difficult to see on the pdf. Based on the location and dendritic morphology of these cells, we believe that the evidence that they are microglia is reasonably strong. Nonetheless, we have softened the language in the results section to reflect the reviewer’s concern.

3. The reviewer raises the question of subgroup analysis. We added to the methods and results section a description of the cohort, which was approximately 51% neovascular, 37% early/dry and 12% geographic atrophy. This has been added to the results section. Furthermore, at the reviewer’s request, we additionally compared the genotype and allele frequencies of the selectin SNPs between each subgroup of AMD (dry, geographic atrophy, wet) and controls. We were pleased to discover that one SNP in SELP (rs3917751) produced a p-value that was statistically significant after multiple measures corrections. We adjusted the abstract, results, and discussion to address this result.

We thank the editors and reviewers for their comments on the manuscript and hope that our revised manuscript will be suitable for BMC Medical Genetics.

Regards,

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