Author’s response to reviews

Title: Epigenetic Landscapes Suggest that Genetic Risk for Intracranial Aneurysm Operates on the Endothelium

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Author’s response to reviews:

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Dr. Matteo Pasini
Editor, BMC Medical Genomics

Re: MGNM-D-19-00204 “Epigenetic Landscapes Suggest that Genetic Risk for Intracranial Aneurysm Operates on the Endothelium”

Dear Dr. Pasini:

We are resubmitting the accompanying manuscript for consideration for publication in BMC Medical Genomics. A point-by-point response to the reviewers’ comments is attached to this cover letter.

Genetics play a key role in the natural history of intracranial aneurysms (IAs), but is not fully understood. Research groups, including ours (Tutino et al. PLOS One, 2018), have detected aberrant gene expression profiles in the circulating blood of patients with IAs, leading us to question if the aberrant circulating expression signatures are the result of an interaction between the blood cells and the aneurysmal tissue or if the formation of the aneurysm is due to dysregulated immune/inflammatory processes that might be attributed to genetics. Thus, in
In this investigation, we studied known IA-associated genetic risk loci to determine if they contained (A) differentially expressed genes identified in our previous studies, (B) genes that are relevant to vascular and immune/inflammatory function, and (C) functional, regulatory elements that are predominantly expressed in one cell type.

In this resubmission, we have addressed the comments from each of the reviewers and have made improvements to the manuscript. In particular, we have added in-depth discussions on results of past genome-wide association studies (GWAS) and more recent studies of epigenetic landscapes in intracranial aneurysm. These and additional changes are highlighted in yellow in the revised manuscript.

Overall, we show that functional regulatory elements within the IA-associated risk regions were present to a significant degree in endothelial cells rather than immune cells. Genes potentially affected by each single nucleotide polymorphism (SNP) that showed cellular processes and functions were related to extracellular matrix and protease regulation. These findings imply that known genetic risk factors for IA are more likely affecting the vessel wall than the circulating inflammatory cells. These novel results shed further light on how IA-associated SNPs may affect IA pathogenesis and highlight the importance of investigating noncoding elements in cell-type specific genomes. We believe that this manuscript addresses a timely issue and will be valuable to the audience of BMC Medical Genomics.

Sincerely,

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Response to Reviewer Comments:

Authors: We thank the reviewers for the insightful comments, which have helped to improve the manuscript. On the basis of these comments, we have made several changes, which are highlighted in yellow in the revised manuscript. Below are our point-by-point responses to the comments. Thank you.

Ynte M Ruigrok (Reviewer 1):

The authors write in the Introduction section that "Genome-wide association studies (GWAS) have identified many single nucleotide polymorphisms (SNPs) that occur more commonly in individuals with IAs." However, they do not describe the results of these GWAS not do they cite any references. This should be added.

Authors: Thank you for pointing this out. We have now discussed and referenced major intracranial aneurysm GWAS and some of their findings in the introduction on pages 5-6. This now reads:
“Genome-wide association studies (GWAS) using large cohorts from principally Dutch, Finnish, and Japanese populations have identified many single nucleotide polymorphisms (SNPs) that occur more commonly in individuals with IAs (15-23). In these studies, significant associations were reported at 2q32.1 (PLCL1) (16), 8q11.23–q12.1 (SOX17) (16), 9p21.3 (CDKN2A-CDKN2B) (16), 18q11.2 (RBBP8) (23), 13q13.1 (STARD13) (23), and 10q24.32.12 (23). The most frequently replicated locus has been 9p21.3 at the non-coding RNA, CDKN2B-AS1, which is in the CDKN2B-CDKN2A gene cluster, and has been shown to be a significant genetic susceptibility locus for cardiovascular diseases (24). In a meta-analysis, Alg et al. (25) investigated 66 case-controlled studies that included 32,887 IA patients and 83,683 control subjects and identified 19 SNPs that were significantly associated with IAs, the most replicated of which were at 9p21.3, 8q11, and 4q31.23. Like those in association with other complex diseases or traits (26-29), several IA-risk loci have been found in noncoding regions of the genome, suggesting that genetic risk may operate on functional regulatory elements that influence gene expression, rather than on the structure of the gene product (30).”

The discussion section starts off with an exact repetition of the introduction section. The whole first part of the discussion until paragraph 'Genetic risk in the endothelium of persons with IA' should be omitted.

Authors: We have removed most of these beginning paragraphs. We did keep a small introduction summarizing the main points before discussing 'Genetic risk in the endothelium of persons with IA' to benefit the reader.

A previous studies already investigated the role of intracranial aneurysm-associated single-nucleotide polymorphisms on regulatory DNA elements: Laarman et al. Intracranial Aneurysm-Associated Single-Nucleotide Polymorphisms Alter Regulatory DNA in the Human Circle of Willis. Stroke. 2018 Feb;49(2):447-453. This study should be discussed.

Authors: Thank you for pointing this out. We have now added a paragraph to page 21 in the Discussion where we relate our findings to previous studies (namely, two by Laarman et al.). This section reads:

“Although direct experimental investigation of epigenetic landscapes in IA has been sparse, in a recent study, Laarman et al.(86) performed ChIP-seq on DNA from postmortem human Circle of Willis tissue to identify histone H3K4me1 and H3K27ac modifications in regulatory regions (distal enhancers and active promoters). They then queried if these regions overlapped with 19 known IA-associated SNP regions (from (22, 23)) and found that 7 of them overlapped with active regulatory regions. Three of the SNPs they queried were also investigated in the current study, namely, rs1132274 (on chr20), rs6841581 (on chr4), and rs658595 (on chr12). Interestingly, rs6841581 was found by Laarman et al.(86) to likely affect TF binding (rs1132274 and rs653859 had minimal and no predicted TF activity, respectively). In a follow-up study, Laarman et al.(87) elegantly used chromatin conformation capture technology to identify enhancer targets of 4 known risk loci and confirmed intrinsic enhancer activity via an in vivo reporter assay. These loci included 2 that overlapped with those studied here: chr4:148365339-148414651 (rs 6841584) and chr9:22077085-22125503 (rs10757278). These studies provide compelling experimental evidence of the predicted enhancer activity in regions reported in the present study (particularly for the SNPs mentioned above). However, because Laarman et al.(86, 87) investigated DNA from multiple cell types (from whole Circle of Willis tissue), the
interpretation of our findings should be limited, pending confirmation through experimental validation in specific cell types, such as ECs, obtained from aneurysm tissue.”

Y Jiang (Reviewer 2):

The author conclude from database that known genetic alterations linked to IA risk act on endothelial cell function. These finding is quite different from IA-associated gene expression signatures of circulating blood cells. Maybe because there are less paper published used circulating blood cell. If any, most of paper lack of detail group to differ the higher risk of intracranial aneurysm. Circulating cell can be a secondary response reflecting the presence of IA and indicating risk for IA.

Authors: Thank you for this comment. We agree that these findings are quite different from those of our previous neutrophil expression studies. From the present data, we believe that ECs carry genetic risk more than circulating immune cells do, and (as you point out) the expression changes in circulating immune cells are a secondary response reflecting the presence of the aneurysms. We have expanded this discussion on pages 22-23, which now reads:

“In the present study, none of the IA-associated LD blocks were significantly enriched for histone marks or CTCF binding sites in neutrophils, which further supports our hypothesis. In fact, our results predict that functional regulatory elements in the IA-associated risk regions are present more in ECs, suggesting that genetic risk for IA is more likely to be conferred through the ECs than the immune cells. This is further supported by the Gene Ontology data demonstrating endopeptidase activity/regulation and ECM structural components, which may play significant roles in ECs, rather than immune cells (neutrophils). These results suggest that aberrant expression observed in circulating immune cells of individuals with IA is a secondary response following IA formation and not an indicator of genetic risk for the disease, at least for the 16 SNPs that we investigated. Therefore, the expression changes in circulating neutrophils that we observed could be caused by contact with inflamed aneurysm tissue or activation by chemokines and cytokines released from the aneurysm (92).”