Author’s response to reviews

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

Authors:

Kerry Poppenberg (kerrypop@buffalo.edu)
Kaiyu Jiang (kaiyujia@buffalo.edu)
Michael Tso (mtso@ubns.com)
Kenneth Snyder (ksnyder@ubns.com)
Adnan Siddiqui (asiddiqui@ubns.com)
John Kolega (kolega@buffalo.edu)
James Jarvis (jamesjar@buffalo.edu)
Hui Meng (huimeng@buffalo.edu)
Vincent Tutino (vincentt@buffalo.edu)

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Author’s response to reviews:

September 3, 2019

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 this investigation, we studied known IA-associated genetic risk loci to determine if they
contained (A) functional, regulatory elements that are predominantly expressed in one cell type, (B) genes that are relevant to vascular and immune/inflammatory function, and (C) differentially expressed genes identified in our previous study.

In this resubmission, we have addressed the comments from each of the reviewers and have made improvements to the manuscript. In particular, we clarified the objectives of the study in the Introduction, clarified confusing sections of the manuscript identified by the new Reviewer, and recreated all figures to increase their resolution. These and additional changes are highlighted in yellow in the revised manuscript.

Overall, this paper shows 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) showed that 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,

Vincent Tutino PhD,

Canon Stroke and Vascular Research Center

Research Assistant Professor of Neurosurgery and Pathology

vincentt@buffalo.edu

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.

Y Jiang (Reviewer 2):

From my view, I just need more explanation, more data support your point.

Authors: We are unsure what more explanation Reviewer 2 is requesting. As noted in our previous response, the questions we addressed emerged from our study of peripheral blood cells (neutrophils). Specifically, we wanted to know whether the gene expression signatures we had identified reflected an intrinsic, underlying defect in neutrophil function. The way we chose to do this was to determine whether there was evidence that the genetic risk factors that are known to contribute to intracranial aneurysm (IA) might impinge on neutrophil function. This does not appear to be the case on the basis of our analyses of the broader chromatin
architecture surrounding the IA risk haplotypes in neutrophils. To address your comment in the last review, we added this point to the Discussion (pages 22-23), and further explained that expression changes in circulating immune cells may be a secondary response reflecting the presence of IA. Testing this would require rigorous experimental studies that are beyond the scope of this paper.

As far as the requested additional data, below we present comparisons of a region around the SNP rs1800255 (on chr2, which we investigated in this study) obtained from publicly-available HiC data from 2 cell lines, myeloid THP-1 and HUVEC (available on the 3D Genome Browser maintained by the Yue lab at Northwestern University). In HUVECs, there are clearly defined topologically associated domains (TADs) demarcated by orange and green bars at the bottom of the figure, which were not identified in the myeloid THP-1 cell line. This was true for all 16 SNPs we studied. The fact that there are regulatory regions in TADs in HUVECs but not myeloid cells further supports the main conclusion of our paper. This figure is part of a larger analysis of chromatin structures surrounding the IA risk haplotypes that will be the subject of another paper from our group.

Reviewer 2 (Reviewer 3):

A clear objective is needed. Perhaps, the author can separate the objectives into main objective and specific objectives.

Authors: Thank you for pointing this out. As you suspected, our study had two objectives. These were 1) to investigate chromatin features in IA-risk SNP regions, and 2) to determine if any of these regions contained genes we reported to be differentially expressed in neutrophils from patients with IA. We have now clarified these objectives in the Introduction on pages 6-7 of the revised manuscript. The last paragraph of the Introduction now begins:

“The overall objective of this study was to gain insights into the pathobiology of IA by examining the chromatin features in genetic regions known to confer risk for aneurysm within pathologically relevant cells. Our secondary objective was to determine if genetic variation in any of these regions could affect gene expression differences reported in our previous neutrophil transcriptome profiling study.”

The resolution of the figures and supplementary figures need to be improved.

Authors: Thank you for this comment. We have recreated all images reported in the manuscript. They are now presented in a resolution of at least 300 dpi.

The author needs to proofread the manuscript as it contains confusing sentences/ hanging sentences as well as typos and grammatical mistakes. e.g.:

1) 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).

2) These loci included 2 that overlapped with those studied here: chr4:148365339-148414651 (rs 6841584) and chr9:22077085-22125503 (rs10757278).

3) ....study (particularly for the SNPs mentioned above).
4) We determined whether any of these differentially methylated regions correlated with the IA-risk LD blocks.

5) Our analyses showed that functional regulatory elements within the IA-associated risk regions were present to a greater degree in ECs compared to immune cells, suggesting that genetic risk for IA is more likely to be conferred through the ECs than immune cells."

Authors: Thank you for catching these confusing sentences and typos. We have gone through the entire manuscript to correct any spelling and grammatical mistakes. We have also corrected the confusing sentences referenced above (pages 11, 17, and 21).