Author’s response to reviews

Title: Progressive Optic Nerve Changes in Cavitary Optic Disc Anomaly: integration of copy number alteration and cis-expression quantitative trait loci to assess disease etiology

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RE: D-18-00425

Full Title: Progressive Optic Nerve Changes in: integration of copy number alteration and cis-expression quantitative trait loci to assess disease etiology

Dear Dr. Pasini,

We are most appreciative and thank you for your positive and encouraging feedback. We also appreciate the reviewers' careful reading and helpful feedback. We include our detailed responses to the reviewer's comments below with tracked changed in the accompanying manuscript.
Reviewer reports:

Takeshi Iwata, Ph.D. (Reviewer 1):

This manuscript adds novel information about CODA, another optic nerve related disease by copy number alteration (CNA). Authors have characterized CNA at accuracy never done before. It would be nice if authors can demonstrate gene expression changes in brain and nerve related genes in Figure 5.

We are most appreciative and kindly thank the reviewer for their positive feedback. We regret however that we do not have brain or the appropriate nerve samples on which to perform gene expression profiling to determine the effect of the CNA on expression of the genes regulated by eQTLs at this time. This would be a great idea for a future experiment.

Minor correction:

Page 9, line 11-13 - 1.1 bp product

We changed it from kb to bp. Thank you for catching this error.

Page 13, line 8, p published

We removed the extra letter “p.”

Subbaiah Krishnadas (Reviewer 2):

There are following queries in the manuscript which require clarification from the Authors:

1. Among the fourteen unaffected family members 5 were carriers of the disease (Male: female ratio- 3:2). Why the copy number variation is high among the 4 carriers than CODA affected members and explain using in silico analysis the specific gene involvement in the regulation among the four genes for the difference in the copy number.

We thank the reviewer for helping us to clarify this. Results from the copy number assay showed that affected individuals had a range of 2.8-4.1 copies compared to 1.5-2.3 copies in unaffected individuals. Copy number assay results were repeated more than three times and found to be affected by sample-specific error, the source of which is unclear. Discussion with customer support indicated that distinguishing between 2 and 3 copies is likely at the limits of the precision of the assay. Clearly a number such as 2.3 copies is likely not physiological since there can only be integer or whole numbers of copies of a given DNA sequence. Therefore, this was the scientific justification used to pursue long-range PCR to more reliably and accurately assay
the number of copies, which was found to be 4 total copies in all affected individuals and the 5 carriers. The manuscript has been revised to include the following statement on page 10, line 8.

"The results of this assay were affected by sample-specific error, the sources of which are unknown."

For the second part of the reviewers’ comment regarding in silico analysis and the specific gene involvement in the regulation among the four genes for the difference in the copy number. We realized the purpose of the in silico analysis was likely not made entirely clear therefore we have changed the sentence under methods to state: “The functional relevance of the copy number alteration region was assessed by in silico analysis”. We have removed the word “genotype” which may have added to the confusion.

2. In the six generation pedigree, mostly females are affected and two of females are carriers. Even though CODA is autosomal dominant, Is there any explanation for the cause in female or any nuclear and mitochondrial genome cross talk for the pathogenesis?

The reviewer makes an interesting suggestion. We also noted the predominance of female gender in affected individuals in our pedigree. We appreciate the reviewer's suggestions and have added the following paragraph to the discussion:

"We observed that although the CNA localizes to an autosomal chromosome, there are more affected females than males in our pedigree as well as the other well characterized CODA pedigrees described to date.1,3,20 Females comprise 66% of the 53 affected individuals described in four CODA pedigrees. This may be due to an abnormal secondary sex ratio rather than sex-specific penetrance, since females comprise 70% of the 71 offspring of affected females in these pedigrees. MMP19 and GDF11 are expressed in the endometrium,14 and although our CNA was not found to be an eQTL for these genes in the uterus, they could potentially affect secondary sex ratio by mediating fertilization, implantation or embryonic death. Different intrauterine conditions are hypothesized to explain the abnormal sex ratio in offspring of carriers of the gene responsible for x-linked retinoschisis.25"

3. Methods-Through interview with 18 living family members, we identified deceased individuals who had adult-onset bilateral vision loss and marked these individuals as affected on the pedigree. Without definite evidence or documentation of clinical features, how is it possible to include the deceased as affected based on retrospective history of bilateral adult vision loss alone- there are several other causes of adult onset bilateral visual decline
The reviewer makes an excellent point. We agree with the comment and revised the manuscript to clarify as following on page 4, line 17 and page 20 line 22 (figure legend) and modified figure 1.

"Through interview with living family members, we identified two deceased individuals who had bilateral vision loss in their 40s and marked these individuals as presumably affected on the pedigree."

We also added a section on author contributions to the declarations. Thank you again for the opportunity to submit this revised manuscript.