Author's response to reviews

Title: Novel VPS13B Mutations in Three Large Pakistani Cohen Syndrome Families Suggests a Baloch Variant with Autistic-Like Features

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Author's response to reviews: see over
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Dear Editor,

Please find enclosed a copy of our revised manuscript (MS: 1464052114152294) entitled: “Novel VPS13B Mutations in Three Large Pakistani Cohen Syndrome Families Suggests a Baloch Variant with Autistic-Like Features”, which we would like you to reconsider for publication in your journal as an Research Article. We have revised the manuscript (highlighted by track-changes”) based on the comments from the two Reviewers and the Editor, and provide a point-by-point response to all the concerns in the following pages.

To our best knowledge this is the second report on VPS13B mutations segregating in large families from different regions of Pakistan. Of particular note, one mutation is present in two large families, both of Baloch origin but from distant parts of the country. Haplotype analysis suggests a founder mutation ~50 generations ago, and thus this may represent a common Cohen syndrome mutation among the Baloch ethnic group. Also of interest, the affected individuals in these two families show autistic-like traits, and thus this mutation may lead to an autistic-like subclass of Cohen syndrome.

We have addressed all the comments from the two Reviewers and the Editor. We believe these findings will be of great interest to your readership, and to the scientific community. We look forward to your final decision.

Yours sincerely,

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Response to Reviewers and Editor:

**Reviewer 1:**

*Major comments*

1. *Please provide the MR Images as Supplemental file. This would improve the quality of the manuscript.*

Brain MRI of ATM02 V-5 has been added to the supplementary materials Figure 3.

2. *The autism phenotype in the ATM02 family is interesting, however, similar observations have been made in context of Cohen syndrome (Ionita-Laza I. et al. 2014 Plos Genetics and Yu TW et al. 2013 Neuron). Those results from the literature should be implemented in the discussion of the manuscript.*

We agree with the reviewer and have added some details regarding the mutations found in individuals with ASD in publications by Ionita-Laza I. et al. and Yu TW et al, and integrated with our results in the discussion on p.17-18, lines 391-418.

3. *In general, please remove the statements about the transmembrane domains in VPS13B. It has been shown biochemically that VPS13B is a soluble protein without any transmembrane domains. Therefore, any predictions about mutations affecting those are not helpful.*

We agree with the reviewer and removed all the statements about the transmembrane domains in VPS13B on p.15 and p.16.

*Minor comments*

*Overall, please check again carefully for minor grammatics and spelling errors: e.g. line 72 “IDdisorder”.*

We thank the reviewer for identifying the minor grammatics and spelling errors. We have carefully proofread the manuscript and corrected all such errors, highlighted by track-changes.
Reviewer 2

Major Compulsory Revisions

(1) - Cohen syndrome is a rare genetic condition which, until now, has not been easily diagnosed, mainly because the size of the responsible gene (VPS13B). In fact there are less than 1000 diagnosed cases worldwide. Nevertheless, the authors state that this syndrome is one of the most common genetic syndromes, right after Fragile X with a frequency of 0.7%. The authors should provide a real incidence of this syndrome, since this 0.7% value was from a study of 670 patients to whom target sequencing was performed.

We have re-phrased the sentence about the incidence of Cohen syndrome on p.7 as follows, which was the conclusion from the referred study:

"In a cohort of 1170 unselected patients referred for developmental delay or ID, Cohen syndrome [COH1][MIM# 216550] was found among several common genetic diagnoses for ID; and with a frequency of 0.7%, COH1 is ranked right after Fragile-X syndrome (1.2%) among patients with clear diagnosis of ID [2].

The world registry of Cohen Syndrome (http://cohen-syndrome.org/) has estimated that globally fewer than 1000 cases of Cohen syndrome have been reported. However, we think Cohen syndrome might have been previously under-diagnosed, especially for those with less or milder dysmorphic facial features, and particularly in populations with high consanguinity. Several high throughput whole exome sequencing projects (Ionita-Laza I. et al. and Yu TW et al, and our current study) have identified many more patients carrying mutations in the VPS13B with variable phenotypic features, and previously diagnosed as ID, ASD or PDD. In the three large Pakistani pedigrees alone we reported in this study, there would be about 30 potential patients compatible with the genetic diagnosis of Cohen syndrome, according to the family history and available clinical information. Therefore, we agree with the conclusion from the previous report by Rauch A et al (2006) that Cohen syndrome is one of the most common, but widely under-diagnosed mental retardation syndromes, as well as in a small percentage of recessive ASD cases.

(2) - The authors decided to perform a homozygosity mapping to interrogate samples even with highly inbred families. The expected number of homozygous regions must be huge. Can the authors provide this information? The final diagnosis is achieved by Whole Exome or Sanger sequencing. In families ANMR51 and RQMR10, did they sequence the VPS13B gene due to the findings of the homozygosity mapping?

Yes, due to the consanguineous marriages, each individual in these families carries a large amount of runs of homozygosity (ROHs), and the affected individuals share multiple HBD (homozygosity by descent) regions. In ATM02 family, the three affected
brothers and their sibling carry an average of 140 ROHs larger than 500 Kb, the average total size of their ROHs is 310,420 Kb. However, three affected brothers in this family only share 4 HBD regions, which are not shared by other unaffected family members and population controls. Since the clinical diagnosis was not clear prior to the molecular genetic study, we have chosen to exome sequence two affected individuals in order to exclude any other potential causative mutation. We have analyzed the exome variants separately and jointly in the two affected individuals. Although, each exome dataset contains more than 20,000 exonic variants plus a small number of potential splicing variants, none of the two affected individuals carries any additional loss of function variant(s) in a gene known to cause autism or ID (SFARI gene list, Dec 2014). We have revised the corresponding paragraph on p. 14.

As in families ANMR51 and RQMR10, although there are multiple ROH regions in each individual, we identified only one shared HBD region (this is really where the advantages of HBD mapping in larger families with multiple affected individuals becomes apparent) on chr8 in each of the two families; therefore directly Sanger sequenced the VPS13B gene based on the findings of the shared HBD in these two families together. We have emphasized this on Page 13 (line 271-272): “We identified only one ~25.430 Mb homozygosity-by-descent (HBD) region shared by the affected individuals, but not unaffected family members…..” and on Page 13 (line 283-284): “Analysis of genotypes for family ANMR51 revealed only one ~4.413 Mb HBD region shared by the affected individuals, but not unaffected family members”.

(3) - The authors mix the description of clinical findings from families RQMR10 and ANMR51, both in the material and methods and the results section. It would be easier to read if they separate the families.  

We have moved and divided the clinical features of families RQMR10 and ANMR51 in the results section only.

(4) - The authors state that most of the individuals with the 312 c.6879delT mutation in two of these families also present with autistic like traits, which suggests that this variant may lead to a distinct autistic-like COH1 subgroup. This conclusion seems too daring for me. Since these families are consanguineous, it cannot be discarded the presence of other homozygous mutations that could be responsible for the autistic features. Has this possibility been evaluated? In two members of the ATM02 family, they have performed whole exome sequencing; maybe they can extract some information from these data.

We agree with the concern of the reviewer and have carefully re-evaluated the exome sequencing datasets for two affected individuals in ATM02 separately and
jointly, for both homozygous and heterozygous variant (for potential de novo variants), particularly for genes outside of the shared HBD regions. We did not find any additional loss of function variant(s) in a gene known to cause autism or ID (SFARI gene list, Dec 2014) and HGMD (Dec 2014). Therefore, we do believe that the 312 c.6879delT mutation is most likely the cause for the autistic features in the affected individuals in these two families.

Minor Essential Revisions
(1) - Authors could specify that the mutations are in homozygosis. e.g Sanger sequencing analysis of all PCR amplicons revealed a homozygous deletion of 1bp.

We agree with the reviewer and have added the word “homozygous” before the variants reported in the paper.

(2) - In table 1, authors should provide data from the percentile and SD of the OFC (cm).

Since the clinical investigations of these families were retrospective, and the ages of the affected individuals at investigation in these three families are in a wide range, from 7 years old to over 50 years old, both males and females. We considered it might be even more confusing to provide the percentile and SD of the OFC for so many different age groups for both sexes. Also, normative data for adults from this population is not currently available, and thus SD cannot be calculated relative to the local population. Therefore, we chose to present the actual measurements.

(3) - Also in table 1, the abbreviation “n.k.” is not used in the table.

n.k. was deleted from the top of the table.

Editor’s Comments:

“I also have a few issues to comment to authors. One major comment is regarding individuals V-1 and V-2 in RQMR10 family, what is the cause of the phenotype in those two patients? If they parents are not related (as seen in the pedigree), it’s very unlikely that they share the same homozygosity region with their children? can authors clarify this point?”
Although we don’t have documentation of consanguinity for the parents of V-1 and V-2 in RQMR10 family, since all marriages in this rural community take place strictly within the clan system, they are almost certainly related, and, if not first cousins, are likely to be at least second cousins. We have added information to this effect in the paper, within the legend for Figure 1: “. For family RQMR10, although the degree of relatedness between the parents of V-1, V-2, and V-3 could not be established, marriages within this rural community are strictly within the clan system, and thus they are almost certainly related”. Since V-1 is deceased, we don’t have genetic or clinical information that would confirm the mutation or Cohen-related phenotype.

“The link of this variant to autistic features does not seem to be enough supported from the data presented. Since many other possible origins can be argued in such inbred families, I would avoid making direct connections between the two observations and would also bring in the discussion the two manuscripts recommended by the second reviewer”

Please see response to Reviewer 2 for the same concern.

“Without providing any additional data from these specific patients, I would also recommend removing the reference to the different degree of NMD for the mutations as it is not proved at all. Specially I would not link the phenotypical differences between individuals from the different families, as their genetic background, consanguinity and other factors might explain such differences”

We agree that NMD has not been proven for the families, however we believe this nevertheless could provide a plausible molecular explanation for some of the variation seen between affected individuals. However, we have changed the sentence, replacing “particularly” with “including” (Page 17, line 389) to “However, the affected individuals from these three families showed remarkable phenotypic variations, which could be due to other factors, including the degree of NMD that may occur for the two mutations, which in itself may depend on the nucleotide context of the premature stop codon and the assembly of the NMD complex”, in order to remove emphasis on this as a strongly indicated explanation.