Author’s response to reviews

Title: First case report of Cohen Syndrome in the Tunisian population caused by VPS13B mutations

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First case report of Cohen Syndrome in the Tunisian population caused by VPS13B mutations

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BMC Medical Genetics

Dear Editor

Thank you very much for considering our manuscript and for the constructive comments. Please find enclosed:

- The revised manuscript (no: MGTC-D-17-00077) in which we addressed all comments of the referees
- The responses to the referee’s comments.

We hope that this revised version meets the referees and Editorial Board standards and is now suitable for publication in the BMC Medical Genetics journal.

Best regards.

Imen REJEB

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Technical Comments:

1. Consent for publication

Please clarify whether you obtained consent for publication also for the accompanying images.

Yes we have obtained consent for publication also for the accompanying images.

Reviewer reports:

Reviewer 1:

1- in abstract, case presentation: authors should add the pigmentory retinopathy found in the girl; a clinical sign important in Cohen syndrome.

We apologize for this omission, as suggested by the referee we added the pigmentory retinopathy found in the girl in abstract, case presentation as follows:

“The proposita presented also pigmentory retinopathy.”

2- in the paper, case presentation: authors should add testing of neutropenia, it is important to know if these patients present neutropenia or not even after the WES was performed. Neutropenia is a clinical sign important in Cohen syndrome, if these patients after confirmation at the molecular level do not present neutropenia, it will add value to the paper.

As suggested by the referee we added in case presentation a paragraph describing the neutropenia tested in the patients in the revised version as follows:

“After the identification of the causal mutation, the reverse phenotyping showed moderate neutropenia and mild neutropenia in the proposita and her brother respectively. In fact, neutropenia is one of the important clinical signs of CS, but because of its intermittent pattern and absence of clinical consequences, it is rarely identified.”
3- in genetic testing: authors should add briefly the technology used for exome sequencing, which Platform? which Bioinformatics Tools were used? how many variants were detected? how did they filter variants to obtain these excellent results?

As suggested by the referee we added in genetic testing a paragraph describing briefly the technology as follows:

“Library generation, exome enrichment and WES were performed at the French National Centre for Genotyping (CNG, Evry, France). Briefly, libraries were prepared from 3 μg genomic DNA extracted from whole blood using an optimized SureSelect Human Exome kit (Agilent) following the manufacturer's instructions. Captured, purified and clonally amplified libraries targeting the exome were then sequenced on a HiSeq 2000 (Illumina) according to the manufacturer's recommendations. Sequence reads were aligned to the human genome (hg19 assembly) using BWA software. Downstream processing was carried out with the Genome analysis toolkit (GATK), SAMtools and Picard Tools (http://picard.sourceforge.net/). Single-nucleotide variants and indels were subsequently called by the SAMtools suite (mpileup, bcftools, vcfutil). All calls with a read coverage ≤5× and a Phred-scaled SNP quality of ≤20 were filtered out. Substitution and variation calls were made with the SAMtools pipeline (mpileup). Variants were annotated with an in-house Paris Descartes bioinformatics platform pipeline based on the Ensembl database.

Different genetics models of inheritance (de novo, recessive, X-linked) were used to perform familial analysis of trio’s exome data. Variants affecting coding regions and essential splicing sites were selected and all variants with a frequency greater than 1% according to several genomic databases (dbSNP: http://www.ncbi.nlm.nih.gov/SNP/, 1000 Genomes: http://www.1000genomes.org/, Exome variant server: http://evs.gs.washington.edu/EVS/ and local platform database) were excluded. All relevant variant were visually explored with Integrative Genomics Viewer (IGV: http://software.broadinstitute.org/software/igv/) to detect false positive results.

With this method and these filters 9 variants were detected in index case (7 with de novo model of inheritance and 2 in the same gene with recessive model). 5 out of the 7 “de novo” variants appeared inherited from one parent (IGV), the 2 others were absent in the affected brother. The 2 variations in VPS13B gene (identified by recessive model of analysis) were also present in the brother; the c.3582delT, p.A1194fs were inherited from the mother and the c.6295_6296delAT, p.M2124fs one from father.”

Reviewer 2:

1- The authors made the effort of reviewing literature but the paper describing glycosylation defects (Hum Mol Genet) is lacking.

We apologize for this omission. The lacking paper was included in the revised version and in order to integrate the glycosylation defects data in the discussion, we added the following paragraph:
“Recent studies have concluded that VPS13B mutations, responsible for COH1, are associated to a tissue-specific major defect of glycosylation and endosomal–lysosomal trafficking defect. This highlights that VPS13B has an important role in Golgi glycosylation and morphology, as well as in lysosomal–endosomal pathway maintenance [8].”

2- Some minor comments: L22 delete « in » L26 « hypotonia » P3L14 delete gene P4L6 "The parents gave their consent "should take place elsewhere Two patients From a non-consanguineous "At least" delete L33 When reevaluated at 12 y, growth retardation and progressive microcephaly were noted P5L1 Her brother L52 was validated P6L1 were of biparental origin L19 delete extremely.

Many thanks for these comments; they were corrected in the revised version.