Author’s response to reviews

Title: Broadening the phenotype of the TWNK gene associated Perrault syndrome

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Author’s response to reviews:

Imen Chakchouk (Reviewer 1):

Author should include the Variant frequency from GnomAD as an extra proof of the pathogenicity of the variants beside the bioinformatic predictions. Only one c.1196A>G allele has been reported in east Asian population. c.1358 G>A was reported once in the European non-Finnish.

We provided the variant frequency for both alterations. The c.1196A>G (N399S) (rs863223921) mutation was found in the gnomAD (MAF: 3.978e-06) and TOPMed (MAF: 0.00003) databases, while this was not present in the 1000Gp3, ALSPAC, ESP6500 and ExAc databases.

The c.1358 G>A (R453Q) (rs760988188) mutation was found in the gnomAD in non-Finnish European the allele frequency is 8.79e-06, TOPMed (MAF: 0.00001), and ALSPAC (2.59e-04) databases, while this was not present in the 1000Gp3, ESP6500 and ExAc databases.

We modified the following part of the text (page 7, first section):
The variant frequency for both alterations are the following: the c.1196A>G (N399S) (rs863223921) mutation was found in the Genome Aggregation Database (gnomAD) in non-Finnish European with MAF: 3.978e-06 in the Trans-Omics for Precision Medicine (TOPMed) database with MAF: 0.00003, while this was not present in the 1000 Genomes Project Phase 3 (1000Gp3), and Exome Aggregation Consortium (ExAc) databases. The c.1358 G>A (R453Q) (rs760988188) mutation was found in the gnomAD in non-Finnish European with MAF 8.79e-06, in the TOPMed with MAF: 0.00001, while this was not present in the 1000Gp3 and ExAc databases.

- Author should also add the ethnicity of the patient previously reported that carried the variant c.1196A>G.

For your request we modified the following part of the text (page 8, first section):
One of the disease-causing mutations, the c.1196A>G (Asn399Ser) variant was already associated with Perrault syndrome by Demain et all in a Caucasian family (Demain et al. 2016).

-I also have one simple question, why are you still using hg18? (Page 5, second section)
We were using the hg38, we are very sorry the hg18 was a typo, we corrected it to hg38.
Now the text is the following:
The sequence was compered to human reference genome (hg38) by NCBI Blast software.

-Add labels to the figures (A, B, C and D)
We added the labels as you requested. The new figures are uploaded to the website.

Diana Cornejo, Ph.D (Reviewer 2):

In the first paragraph, precision should be made that the gene is not located in the mitochondria but in the nuclear genome (chromosome 10). However the protein product does act in the mitochondria.

Thank you for your comment. We modified the text to reflect the position of the gene and the protein clearly. Now the text is the following (page 2, first section):

TWNK (previously C10orf2) is a gene (chromosome 10) encoding the Twinkle protein, an adenine nucleotide-dependent DNA helicase acting in the mitochondria, with a focal function in the maintenance of the mtDNA integrity (Tyynismaa et al. 2004)(Milenkovic et al. 2013).

Attention should be made throughout the manuscript regarding the acronyms that should be explicitly mentioned before appearing in the text.

Thank you for the remark, we modified the text at the following parts:

In Perrault syndrome, besides the central nervous system (CNS) symptoms, sensorimotor peripheral neuropathy is an important and frequent finding. (page 3, fourth section)
Both axonal and demyelinating forms of neuropathies have been described by NCS (nerve conduction studies) (page 3, fourth section)
EEG (electroencephalography) detected mild cortical dysfunction. ENG (electroneurography) observed severe mixed type neuropathy. (page 6, first section)
An NGS (next generation sequencing) panel of 51 genes responsible for the intergenomic communication revealed a compound heterozygous mutation (page 7, second section)

A reference should be added in this part "In ad-PEO3, mild cortical atrophy and white matter abnormalities in subcortical and periventricular regions were described.” We added the missing reference as requested (page 3, third section)
In ad-PEO3, mild cortical atrophy and white matter abnormalities in subcortical and periventricular regions were described (Oldak et al. 2017).

POLG1 gene was Sanger sequenced, however there is no information in the background that could explain why this gene was analyzed
POLG1 gene mutations are associated with very broad phenotypic spectrum and are a very common cause of mtDNA maintenance disorders. This was the reason why after detecting the mtDNA deletion we investigated at first this gene. At that time, we did not have NGS in the institute, so the genes were investigated by Sanger one after the other.

It is not clear to me why hg18 was used, since there is a more complete human genome version such as hg19 or the later hg38
We were using the hg38, we are very sorry the hg18 was a typo, we corrected it to hg38.
Now the text is the following:
The sequence was compared to human reference genome (hg38) by NCBI Blast software.

It would be interesting to give an annex table indicating the 51 genes that conform the used panel
We added an Annex Table with the following contents:

Annex Table 1.
The list of genes investigated by the NGS panel:
AARS2, APEX1, ATP5A1, C10orf2, C12orf65, DARS2, DGUOK, EARS2, ERCC6, FARS2, GFM1, HARS2, IARS2, LRPPRC, MARS2, MFN2, MGME1, MICU1, MICU2, MPV17, MRPL3, MRPS16, MRPS22, MSTO1, MTFMT, MTO1, MTPAP, POLG, POLG2, PUS1, RAD51, RARS2, RHOT1, RHOT2, RMND1, RRM2B, SARS2, SCO1, SLC25A3, SLC25A4, SUCLA2, SUCLG1, TACO1, TFAM, TK2, TRMU, TSFM, TUFM, TYMP, WARS2, YARS2

Indicate which was the pipeline used to filter and analyze the sequence data, and as a recommendation variant classification should follow the ACMG guidelines for pathogenicity
Analysis and variant calling was performed with SureCall software (Agilent Technologies, CA, USA). We filtered for known disease-causing/benign variants (HGMD, Alamut, dbSNP, ensembl.org, PubMed, RGD, ftp.expasy.org, DMDM databases) and for rare variants (minor allele frequency &lt;0.5% in Exome Aggregation Consortium (ExAC )and 1000Genomes (1000Gp3), Genome Aggregation Database (gnomAD) and Trans-Omics for Precision Medicine (TOPMed) databases. Mutations were filtering by prediction softwares (Polyphen2, SIFT, MutationTaster). In the analysis we followed the below listed main steps: First, we set the following quality scores: GATK (QUAL) &gt; 50, GQ&gt;40, RD&gt;4. We used the SureCall software to filter the variants. The missense, nonsense, indels and splice site variants were
selected with a MAF of <0.01. All SNVs with higher score than three of SureCall were excluded. Next filtering was performed with in silico prediction software (Polyphen2, Mutation taster: FATHMM). The confirmation and segregation analyses of the alterations were performed by Sanger sequencing. Finally we used the ACMG guideline to indicate pathogenicity of the variants.

We included all this information in the text of the manuscript:

Library preparation was performed with SureSelect QXT kit (Agilent Technologies, CA, USA) according to the manufacturer’s instructions. Panel: 98 191 kbp region size, 1358 probes. Sequencing: MiSeq reagent kit v2, 300 cycles, Illumina.MiSeq platform (Illumina, San Diego, USA) was used for the NGS run. Analysis and variant calling was performed with SureCall software (Agilent Technologies, CA, USA). We filtered for known disease-causing/benign variants (HGMD, Alamut, dbSNP, ensembl.org, PubMed, RGD, ftp.expasy.org, DMDM databases) and for rare variants (minor allele frequency <0.5% in Exome Aggregation Consortium (ExAC )and 1000Genomes (1000Gp3), Genome Aggregation Database (gnomAD) and Trans-Omics for Precision Medicine (TOPMed) databases. Mutations were filtering by prediction softwares (Polyphen2, SIFT, MutationTaster). In the analysis we followed the below listed main steps: First, we set the following quality scores: GATK (QUAL) > 50, GQ > 40, RD > 4. We used the SureCall software to filter the variants. The missense, nonsense, indels and splice site variants were selected with a MAF of <0.01. All SNVs with higher score than three of SureCall were excluded. Next filtering was performed with in silico prediction software (Polyphen2, Mutation taster: FATHMM). The confirmation and segregation analyses of the alterations were performed by Sanger sequencing. Finally we used the ACMG guidline to indicate pathogenicity of the variants.

Specify which members of the family underwent Sanger screening for the mutations. Where the siblings also tested?
The father, the mother and two healthy siblings were tested as indicated in the new Figure that was requested (see in an answer to a following remark).

FATHMN, is FATHMM just correct the typo
We corrected the typo.

I think is useful to see the pedigree information with segregation information and Sanger traces of the identified mutations
We added a new Figure (Figure 4.) to show the requested information. We uploaded the Figure to the website. Here we are presenting the new Figure:

Figure 4. 4A. Sanger traces of the identified mutations 4B. Pedigree information with segregation information. The mother is heterozygous for c.1196A>G, the father is heterozygous for c.1358A>G, and one female sibling is heterozygous for c.1196A>G, the other healthy sibling does not carry any of the examined mutations

Denote the transcript used to annotate the mutations as well as the corresponding protein change.
For your requested we updated the following sentence (page 6, third section):
An NGS panel of 51 genes responsible for the intergenomic communication revealed a compound heterozygous mutation (c.1196 A&g;G, (p.Asn399Ser)(rs863223921), c.1358 G&g;A, (Arg453Gln) (rs760988188) in the TWNK gene (NM_021830.5).

Where the mutations screened in the unaffected siblings?

Yes they were screened in the two unaffected sibling. One do not carry any of the mutations examined, the other is a carrier of the c.1196A&g;G mutation. We added a new figure with the pedigree information and uploaded to the website as indicated in a previous answer.

What is the frequency of the mutations in the ethnic-matched population?

We added the frequency information to the text, now it is the following (page 7, first section):

The variant frequency for both alterations are the following: the c.1196A&g;G (N399S) (rs863223921) mutation was found in the Genome Aggregation Database (gnomAD) in non-Finnish European with MAF: 3.978e-06 in the Trans-Omics for Precision Medicine (TOPMed) database with MAF: 0.00003, while this was not present in the 1000 Genomes Project Phase 3 (1000Gp3), and Exome Aggregation Consortium (ExAc) databases. The c.1358 G&g;A (R453Q) (rs760988188) mutation was found in the gnomAD in non-Finnish European with MAF 8.79e-06, in the TOPMed with MAF: 0.00001, while this was not present in the 1000Gp3 and ExAc databases.

I think this paper adds important information about the phenotypic spectrum of this rare disease with clinical, imaging and neuropathological characterization.

I only advise careful interpretation of likely pathogenic mutations based on the ACMG guidelines.

We completed the interpretation of the variants based on the ACMG guidelines and we indicated it in the text as requested in a previous remark.

Both the previously published variant the c.1196 A&g;G, and the novel c.1358 G&g;A variant described by us are likely pathogenic based on the ACMG guideline (both PM2, PP1, PP2, PP3, PP4). This sentence is built in on Page 7.