Reviewer’s report

Title: New PCNT Candidate Missense Variant in a Patient with Oral and Maxillofacial Osteodysplasia: A Case Report

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Reviewer: Gerard Tromp

Reviewer's report:

The manuscript describes and interesting case report of a patient with local osteodysplasia, with the identification of a missense mutation in PCNT, a candidate gene for genetic skeletal disorders. A major concern is that there is no comment on how the heterozygous mutation could result in local osteodysplasia, when homozygous null mutations have previously been shown to be the cause of MOPDII. What is interesting is that it is local and not systemic osteolysis; why? Given the role of PCNT is the centrosome and cell division, its role in primary dwarfism is conceivable. Why does the current mutation have no effect during development and manifest itself only in adulthood and only locally? What is the proposed mechanism of action, haploinsufficiency or dominant negative effect? These questions must surely have presented themselves to the authors. Even if they cannot provide an explanation, the questions should be acknowledged as highly puzzling.

There are numerous problems in the manuscript.

The phrase "The relative affordability and accessibility of genome-wide sequencing have facilitated the development of ..." is directly plagiarized from Wright et al., Genetics in Medicine. 20:1216 (2018). The authors are advised to rephrase this in their own terms.

The authors indicate in the abstract and background sections that they performed whole-genome sequencing, but the methods indicate that they perform whole-exome sequencing. There is an important distinction between the two and the authors should represent what they did accurately.

The authors repeat the coverage statistics in two sections: "DNA library preparation and HiSeq sequencing" and "Gene annotation" (under "Candidate gene approach"). They disagree on average coverage: 137x vs 107x. Also, HiSeq is NOT a sequencing method, it is a specific implementation of sequencing. The title should therefore be "DNA library preparation and sequencing", and HiSeq should be mentioned in the methods as the platform on which the sequencing was performed.
There is no description of how alignment to the genome was performed and which version of genome was used as reference.

There is no description of DNA isolation. Only that QC and subsequent library construction was done by GeneWiz.

How was this variant (mutation) chosen? Was it the only non-synonymous sequence difference between mother and child in the genes included in the skeletal nosology? If yes, this should be explicitly stated. If not, how many other variants were there, and how was this one chosen as the most likely candidate?

The abstract and discussion mention "Polymorphism Phenotyping v2" (this is PolyPhen2), but it is not described in the methods. One further wonders why the authors perform PolyPhen2 analysis separately from that already provided in ANNOVAR. That is, ANNOVAR provides annotation from about 26 tools that predict deleteriousness of variants, including PolyPhen2; it therefore appears redundant and illogical to subsequently rely on PolyPhen2 alone as an indicator of deleteriousness.

When reporting variants in genes that have multiple transcripts, it is useful to report the canonical transcript first. That is, in the case NM_006031 is the canonical transcript and NM_001315529 is a minor transcript. Therefore, the amino acid description C839R should be used as the canonical description.

When describing the MOPDII mutations care should be taken to ensure that it is clear that all are homozygotes or compound heterozygotes for null mutations.

There are also numerous small errors in precision and in language use.


). The van der Auwera reference may, or may not, have been used.

Table 1 is not a differential diagnosis table. At present it is a list of other syndromes to consider during differential diagnosis. It would be more useful if it presented the characteristics of each, highlighting those features that are the same and emphasizing the differences.

When reporting variants, it is not necessary to report them in the same concatenated form that ANNOVAR uses. This format is merely a convenience for computational work, but is both unnecessary and distracting in a manuscript. Please add spaces between components and start with the gene symbol (suitably italicized).

For example:

(line 17 page 9) can be presented as:


Note that in this format, the components will break across lines in a way that remain easily interpretable.

Also note that in the legend to Fig 5, NM_001315529 has been dropped from the concatenated descriptor making the c.T2161C:p.C721R uninterpretable.

Under "Funding" the authors indicate that there was no financial support for this analysis. This leads to the question "who paid for the sequencing?" One infers that the sequencing was a gift from Genewiz. If the inference is correct, it would be more accurate to indicate that "Whole exome sequencing was provided free of charge by Genewiz."

Figures 4 and 5 are of unacceptable resolution. In Fig 5A it is impossible to read the bases and the colours of the sequence trace cannot be distinguished. Fig 5B is a line diagram that should be at least 1000 dpi if rasterized, alternatively it should be a vector graphic. Also, it would be most useful to indicate the start ATG codon. The diagram is misleading since NM_006031has the ATG in codon 1 (LRG numbering) and skips exon 2, whereas NM_001315529 starts at exon 2 (LRG), i.e., is from an alternative promoter, and has the ATG in exon 4. The figure suggests that the transcripts are from the same promoter and exon 2 is skipped in NM_001315529.
Last, there are a large number of language use, grammar and punctuation problems.

"Some type of systemic abnormality" is incredibly vague and all encompassing. Authors should rephrase to have more relevance to the phenotype.

WGS is not beneficial at detecting variants, it is effective at it.

"… without systemic disorder" is vague

One performs sequencing on a patient (preferably a patient specimen), not in a patient; one performs it in a laboratory.

PCNT is a gene symbol, it is therefore redundant to use gene in conjunction with it. One therefore has identified a variant (mutation) in exon 14 of PCNT (Pericentrin). It also should be italicized (see HGNC recommendations) whenever used in the gene context.

A variant does not damage protein structure, it might disrupt, alter or otherwise affect protein structure.

WGS does not result in diagnosis of (rare) disease. Diagnosis remains a clinical activity. WGS identifies variants or mutations that cause or contribute to the disease phenotype.

Change "… youngest of the two children." (line 56 page 4) to "… youngest of two children." The specific article is only use when it has already been identified that there are two and only to children.

When there are embedded parentheses (page 5), it is convention to change the shape of the outer parentheses to square brackets to assist disambiguation. If more levels are necessary, curly braces can also be used.

Change "99mTc bone scintigraphy" to "Technetium (99mTc) bone scintigraphy" throughout for improved readability.

Change "… systemic bone metabolism." (lines 45 and 46, p 7) to "… disturbances in bone metabolism." Bone is a surprisingly highly metabolic tissue; therefore, stating that something is accompanied by systemic bone metabolism conveys no information.

When describing the characteristics of a group (such as a disease) it is inappropriate to state that "individuals have an average of" … Individuals have a specific value for some metric, but the group has an average and standard deviation. On page 8, lines 46 to 49, the sentence should read that MOPD II is characterized by an average birth weight of less than 1500 g at term, an adult height of about 100 cm, and …"
On page 9, line 34, the authors use the word "impact" (description of polyphen2). In this they propagate the inappropriate use of impact by the authors of polyphen2. A variant can have an effect or a consequence, they cannot have an impact (force over time).

The mechanism (of action) on page 9, line 49 is not specific to the patient. Rephrase to mechanism of action of this mutation.

The last paragraph on page 9 is disjointed and awkward. Authors are trying to set up a contrast (re bone biopsy) but make a statement followed by a disjointed however. This needs to be rephrased.

Page 10, line 5. Authors need to clarify "underwent dental maintenance". It is entirely unclear what this means. Could be as little as a cleaning and as much as regular and intensive follow-up to determine if, and when, reconstruction will be necessary."

It is not clear why WGS is in the abbreviations since it is only used incorrectly in the abstract and introduction. Once the manuscript has been correctly altered to reflect that whole exome sequencing was performed, this abbreviation will be unnecessary.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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