Author’s response to reviews

Title: Familial inheritance of the 3q29 microdeletion syndrome: case report and review

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To the Editors:

Re: manuscript MGNM-D-18-00166

Please find our revised manuscript entitled “Familial inheritance of the 3q29 microdeletion syndrome: case report and review” by Khan et al. We have revised the manuscript in accord with the comments and suggestions of the Reviewers as detailed below. Please note that changes in the revised Case Report are identifiable by the ‘track changes’ feature of MS Word. We thank the Reviewers for their comments and believe the manuscript has been improved by the review. We hope the manuscript is now acceptable for publication in BMC Medical Genomics.

Reviewer Comments

Reviewer 1:

The manuscript is concise and well written. The message of the manuscript is not novel, although it might be informative to stress the importance of a molecular diagnosis for families that harbor copy number aberrations with variable expressivity. Reports of the 3q29 deletions inherited from an unaffected parent have been published before.
We appreciate the Reviewer’s thorough critique of our manuscript and positive comments for improvement. Although the 3q29 microdeletion syndrome has been previously reported, the importance of potential familial inheritance and variably expressivity has not explicitly been highlighted in previous publications. As such, we believe that our case report and review of inherited 3q29 microdeletions does provide novelty and an important perspective that can be extrapolated more broadly to other clinical genomic disorders with variable expression.

1. In the Introduction, the authors mention the "recent discovery of several ... syndromes". The references, though, are not recent.

This statement has been updated to reference more recent literature from exome/genome data implicating clinically-relevant copy number changes across a spectrum of neurodevelopmental disorders.

2. "The majority of 3q29 microdeletion syndrome cases occur de novo [8-12]; however, inheritance from a mildly affected parent has also been reported in some families (Table 1) [13]". How many cases? Please also refer to the other papers describing familial cases.

We have updated Table 1 to comprehensively include reported cases of inherited 3q29 microdeletions. We excluded instances where the deletion was proximal to the common 3q29 microdeletion region and did not include known causative genes (e.g. PMIDs: 26620927, 29410707), as this was not the focus of the manuscript. A total of 11 inherited cases, including our reported case, have been incorporated into the revised manuscript.

3. Apparently, the 3q29 deletion is an important risk factor for schizophrenia (~40 fold). Do these schizophrenia patients have (other) features of the 3q29 microdeletion syndrome? This stresses that the penetrance is not 100%.

From the 2015 Mulle et al. meta-analysis on 3q29 microdeletions and schizophrenia risk, individuals that carried 3q29 microdeletions were reported with mild intellectual disability, autism spectrum disorders, epilepsy, mild learning disability, and impaired social interaction (2010:20691406, 2011:21285140). Although some features of the classic 3q29 microdeletion syndromes are found in the population with schizophrenia, the overlapping clinical features are typically milder. This would be more in line with the concept of ‘variable expressivity’ of the 3q29 phenotype rather than incomplete penetrance.

In response to this important comment we have underscored this variable expressivity and pleiotropy when discussing the increased risk for schizophrenia in the revised manuscript.

4. The authors mention that the mother carrying the deletion is healthy, but she does have a history of learning disabilities. What kind of disabilities. Does she still have a subtle cognitive impairment or signs of neuropsychiatric disorders?
The patient’s mother is able to tell us that she had learning disabilities that required her to be in special education classes up through high school, her highest level of education. However, she was not able to give us specific details about her disabilities and did not have any records from her school that could be reviewed. Based on our interactions, the patient’s mother is a very concrete thinker and requires constant reminders about appointments and follow up. However, to our knowledge, she has not been formally seen for a neurological evaluation nor does she have the means financially to obtain such an evaluation. Without a true evaluation, we would not be able to directly comment on subtle signs of cognitive impairment or neuropsychiatric disorders.


The Van Driest et al (2016) reference is correct as it does talk about incomplete penetrance related to previously reported ‘pathogenic’ arrhythmia-related channelopathy sequence variants and a lack of clinical manifestations among many individuals that carried these variants in a biobank consortium population. However, in response to this comment, we have updated the discussion to include an additional reference that supports incomplete penetrance/variable expressivity for clinically relevant copy number aberrations (PMID:28963714).

6. The authors conclude that anemia is an uncommon feature of the syndrome. It might just be a coincidence. It seems too early to tell whether it is a rare feature or not.

We agree with the Reviewer that it is premature to postulate whether this is a rare finding and additional patients would be needed to make this correlation. This sentence has been modified in the revised manuscript and the entry on anemia has also been removed from Table 1 where only the main clinical features are tabulated.

7. Also the statement that "the familial inheritance of this pathogenic deletion is unique" is striking, because in the same sentence other examples are mentioned.

The phrasing has been modified in the revised manuscript, as it was originally written to underscore the smaller size of the 3q29 microdeletion in our study (1.2 Mb) compared to the recurrent 1.6 Mb deletion.

The resolution of the Figures is too low.

We increased the resolution of the Figures in our revised submission and have uploaded them as PDFs. We will work with the Journal as needed to optimize these if the manuscript is accepted for publication.
Reviewer 2:

Khan et al. report a family with a 3q29 microdeletion, slightly smaller than the recurrent microdeletion and present in a child and his affected mother. This report highlights implications of identifying genetic changes that may lead to milder phenotypes - and variable expressivity of phenotypes - in the setting of familial inheritance.

The presentation and review of the literature is currently imprecise and could use clarification.

We thank the Reviewer for the careful review of our manuscript and constructive suggestions for clarification.

Major points:

1. Clearer discussion of the "3q29 microdeletion syndrome" as being defined by the recurrent, 1.6-Mb deletion caused by non-allelic homologous recombination. This should be explicitly stated in the background. While most of the literature cited is about the recurrent microdeletion, there are some reports of other atypical deletions mixed in. The use of the "3q29 microdeletion syndrome" term should be carefully defined/applied, perhaps being limited to cases with the recurrent deletion.

   This is an important comment and we appreciate the Reviewer highlighting the need for clarification of the syndrome in relation to the atypical 3q29 deletions. As suggested, non-allelic homologous recombination as the likely mechanism has been added to the Introduction in the revised manuscript.

   Regarding the second part of the Reviewer’s comment, we used the ‘3q29 microdeletion’ term to refer to both the recurrent and smaller microdeletions (i.e. nested within the 1.6 Mb recurrent deletion), as both aberrations contain landmark genes in the common regions where they overlap, namely DLG1 and PAK2 (also see Figure 2A). This is also mentioned in the discussion section (3rd paragraph) and references therein, (see PMIDs: 18471269, 26055425, 21850710); with DLG1 being implicated in the behavioral and intellectual disability characteristics of this microdeletion syndrome (PMIDs: 20453639, 21850710). These landmark genes also define the common deletion region as was originally described (15918153) and recognized by the larger genomics community (ISCA 37443; https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/clingen_region.cgi?id=ISCA-37443). We outlined this case report to focus on the recurrent deletion and smaller overlapping CNVs such as the one presented here. As such, we have defined the ‘3q29 microdeletion syndrome’ terminology as applied to this case in the Introduction of the revised manuscript.

2. Inheritance. Table 1 reportedly reviews reports of inherited 3q29 microdeletion cases, but it is incomplete. For example, the paper by Clayton-Smith et al. (reference #8) is not included in the table.
Thank you for identifying the additional inherited case from Clayton-Smith et al., which has been updated to Table 1 in the revised manuscript. In addition, in response to this comment and those from Reviewer 1 above, we have again reviewed the literature for familial cases of this microdeletion syndrome. Excluding de novo, inconclusive, or cases where parental follow up was not mentioned (e.g. see PMIDs: 15918153, 16760732, 17124408, 19298871, 20832509, 20830797, 21626679, 23443968, 24214349, 25714563), published data of inherited 3q29 microdeletion syndrome have been updated to Table 1. The other examples of inheritance described in the literature are those of the 3q29 microduplication syndrome which were not included in our study. In addition, truly atypical centromeric 3q29 deletions (PMIDs: 26620927, 29410707) that do not include the commonly deleted genes (e.g. DLG1, PAK2), are not typically considered part of the 3q29 microdeletion syndrome, and were therefore not incorporated into our study.

3. Coordinates. In table 1, there is a mixture of genome builds in the reported coordinates of the deletions. These should all be converted to the same build and reported as such. Additionally, the coordinates are marked as unknown - for example, the inherited deletion from the Ballif et al. is shown in the paper to be the standard, recurrent deletion. (Also, it is not clear where the phenotype data for this report comes from, as patient-level data doesn't seem to have been included in the paper. The mother was also reported as "mildly affected" but is not shown as having any symptoms.)

All coordinates provided in Table 1 have now been lifted over to the GRCh37/hg19 genome build as those from Digilio et al, Petrin et al, and Li et al were reported on the hg18 build. In Monfort et al (2008), the authors used a BAC/PAC clone microarray (older version) and did not report on their genomic interval. FISH was performed with an Abbott subtelomeric probe (3QTEL05; D3S4560) to confirm the deletion. This probe has a sequence tag site D3S4560 corresponding to DLG1 (on older hg17 genome assembly). The size of this deletion is given as 1.2 Mb but since it is an older microarray build, the boundaries may not be clearly defined.

The Reviewer is correct in that the mother of the two siblings in Ballif et al. was mildly affected, and as such details of her ‘mild phenotype’ were previously not mentioned in our original Table and not formally discussed in the Ballif et al study. Regarding the patient-level phenotype for the siblings with the inherited deletion, this was broadly abstracted from Table 1 in Ballif et al. However, the specifics on the phenotype were not provided for these two siblings (likely shown in Fig. 1F and G), but they exhibited the typical 1.6 Mb deletion. For clarity, our Table 1 has been updated to reflect this with the following footnote: ‘the information provided for the siblings from the Ballif et al study reflects clinical features common to their recurrent microdeletion cohort, as further summarized in the 2nd paragraph of their paper’.

4. Atypical deletions. The reported patient and his mother have a slightly smaller deletion than is recurrent. This should be stated earlier when the testing results are presented; it is currently not stated until the discussion or through examination of figure 2. The authors could also consider specifically stating what is the difference between their patient's deletion and the recurrent deletion - what are the genes that are normally deleted that have been spared in this patient? As
these are relatively few genes and not the proposed critical genes, perhaps this family would be expected to have similar manifestations of the recurrent microdeletion syndrome? Additionally, the paper could benefit from a more explicit review of other cases with smaller, atypical deletions within the recurrently deleted region. For example (not necessarily an exhaustive list): Mulle et al. 2014 (PMID 23871472), Cox & Butler (Ref. 9), Dasouki et al. (Ref. 10), and Ballif et al. (Ref. 13 -although detailed clinical information is not provided) all include smaller deletions, several of which seem similar to the deletion in this proband. And while the paper by Cobb et al. refers to a "1.3-Mb" deletion, this was found by BAC array, and not enough information is available to confirm if this is the recurrent deletion or atypical - so it should not be included among cases with smaller, atypical deletions.

The 1.2 Mb deletion in the proband, which was smaller than the recurrent 3q29 microdeletion, is now mentioned in the case presentation of our revised manuscript. The explicit difference between the 1.6 Mb recurrent deletion and the 1.2 Mb deletion observed in our proband is also described in paragraph 3 of the discussion and conclusion sections. The 1.2 Mb deletion reported in our proband does in fact contain the candidate/critical genes that have also been proposed as landmark genes in this region (ISCA-37443).

In response to this comment and those from Reviewer 1, we have added a brief overview of smaller deletions in the revised manuscript. Of note, we do not consider these smaller deletions that relate to the 3q29 microdeletion syndrome region technically as 'atypical deletions' but rather as smaller deletions since they still contain the proposed candidate genes found in the recurrent region. Please note that while smaller deletions were included, truly 'atypical' deletions (e.g. see PMIDs: 26620927, 29410707) that are proximal to the 3q29 microdeletion was not the focus of this case report. As mentioned above, these atypical deletions do not involve the candidate genes, are not flanked by the same segmental duplications and are not as yet considered part of a syndrome. Please note that PMID 23871472 mentioned above was not added as this report is related to a reciprocal Williams syndrome duplication reported by Mulle et al.

Minor points:

1. Abstract, case presentation, and discussion: Missing hyphen in "3-year-old".

Hyphens at designated locations have been added and the manuscript was checked for additional similar formatting changes.

2. Background, 2nd paragraph: The authors mention that the 1 in 30,00-40,000 frequency may be an underestimate, but this number is derived from a population-based screening so wouldn't be subject to an underascertainment bias.

To date, the phenotype of the 3q29 microdeletion syndrome is not well described, especially those with a neuropsychiatric component, as also recognized by the curation efforts of the 3q29 registry (referenced in manuscript -- Glassford et al., 2016, PMID: 26738761). And for this reason, the phenotype could present with a subtler constellation of features in certain families, and therefore missed as it is not clinically overt. Moreover, the frequency of this microdeletion,
although identified through a large Icelandic study (PMID:24352232), did not account for ethnicity stratification and its effect size was not as robust as some of the well-known genetic epidemiological studies with appropriate case controls (2008:17911159, 2014: 25217958). If we were to add to this the complexity of variable expressivity/incomplete penetrance (while high but not 100% as per GeneReviews; https://www.ncbi.nlm.nih.gov/books/NBK385289/) along with platform specific limitations in detection, an underestimation in the incidence of this and other clinically relevant copy number aberrations does indeed begin to emerge (2009:19166990). However, given this concern raised by the Reviewer, we have clarified this statement in the revised Introduction to be inclusive of these limitations.

3. Case report, CMA results: What are the refined coordinates of the deletion, based on the higher-resolution CytoScan testing?

The revised deletion boundaries using Affymetrix CytoScan HD, used as a second algorithm to confirm our findings, were similar to the one detected by the Agilent 180k array and are provided below. These have been updated to Figure 2 legend of the case report as they can be seen in context with the Agilent array.

<table>
<thead>
<tr>
<th>CN State</th>
<th>Type</th>
<th>Chromosome</th>
<th>Cytoband Start</th>
<th>Size (kbp)</th>
<th>Marker Count</th>
<th>Gene Count</th>
<th>Mean Log2Ratio</th>
<th>Microarray Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>Loss</td>
<td>3</td>
<td>q29</td>
<td>1,223</td>
<td>1,265</td>
<td>26</td>
<td>-0.566</td>
<td>arr[hg19] 3q29(195,806,608-197,029,439)x1</td>
</tr>
</tbody>
</table>

4. Discussion & Conclusions, 1st paragraph: "structural variation" is referred to in the first sentence, but it may be clearer to state chromosomal or genomic structural variation.

Collectively, ‘structural variation’ describes both apparently balanced and/or complex rearrangements in the genome on a chromosomal scale as well as copy number variation; however, since we are referring to CNVs, this has been amended as suggested.

5. Discussion & Conclusions, 1st paragraph: it may also make sense to cite a paper like Ref. 3 that attempts to catalog CNVs associated with human disease?

The Coe et al study (2014) corresponding to reference 3 in the reviewed manuscript has been added to the opening sentence of the 1st paragraph of Discussion & Conclusions.

6. Discussion & Conclusions, 1st paragraph: The authors cite a study about penetrance/interpretation of SNVs, but there are additional studies that could be cited that are specific to CNVs. For example, Ref. 24 & PMID 23258348 about penetrance; there is also literature more focused on counseling difficulties (for example, PMID 29146387).
A similar comment was raised by Reviewer 1. As noted above, the 2018 (29146387) reference on interpretative challenges specific to clinically relevant copy number aberrations has been updated to the 1st paragraph of the discussion.

7. Discussion & Conclusions, 2nd paragraph: this paragraph is mostly re-stating much of the information from the background and could be further streamlined.

This paragraph was briefly amended; however, the lesser known features of this syndrome do not come across in the background section so we believe that it is important to underscore these here.

8. Discussion & Conclusions, last paragraph: The authors note anemia as an uncommon finding in the 3q29 deletion. Because it is uncommon, it is possible this is finding unrelated to the deletion, and that possibility should be stated.

The same point was raised by Reviewer 1 and this suggestion has been amended to the last paragraph.

9. Figure 2 legend: Description of panel A could be clarified. The top panel has all of chromosome 3 showing, and the bottom has a "zoomed-in" (as opposed to "zoom-in") view.

Description to panel A in Figure 2 legend has been clarified. The text was also reorganized so the annotations in panel A are described in order of their presentation. ‘Zoom-in’ view was changed to ‘zoomed-in’ view as indicated.

10. Figure 2 legend: Mentions an arrow in figure 2D, but there is no arrow showing.

Thank you for identifying this illustration error. The missing arrow has been updated to Figure 2, panel D.

We expect this Report will be of interest to your readership and we hope that it is satisfactory for publication in BMC Medical Genomics.

Sincerely,

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