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Investigating the genetic aetology of three facial dysostoses in South Africa

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EXAMINER 2 COMMENTS

NOTE: Examiners comments in black and candidate comments in red.

A. Major Comments

1. I think that it is a major omission to not do Sanger sequencing validation of the prioritised variants. If these variants are not real then all the conclusions drawn for this work, are not valid. Also, this was stated as one of the study's objectives. The candidate therefore needs to provide a solid scientific justification (time constraints is not appropriate) as to why Sanger sequencing validation is not necessary for this MSc dissertation. Validation of putative disease-causing variants could not be performed because of time limitations. I appreciate the reviewer feels this is not a valid reason for omitting the Sanger sequencing validation step, and I acknowledge that is was one of the objectives of the study. However, the time to undertake patient recruitment was significant and then the set-up of the NGS methodology took longer than expected as this is a new technique in our laboratory and a lot of trouble-shooting was necessary in the end.

As a point of discussion, it should be acknowledged that while Sanger validation of NGS results is the standard approach in diagnostic laboratories, several studies have suggested that validation of NGS detected variants using Sanger could be unnecessary provided a minimum coverage was achieved for the NGS: In 2013, Sikkema-Raddatz *et al* obtained a 100% (n=168) validation of variants identified through targeted NGS. In this study, it was concluded that an at least 30x NGS coverage is equivalent to the specificity and sensitivity of Sanger sequencing and variants identified through targeted NGS with a minimum of 30x coverage do not necessarily need to be validated (Sikkema-Raddatz *et al*., 2013). All seven putative disease-causing variants identified in this study had a minimum coverage of 37x (See Table 3.3, page 39). Baudhuin *et al*. (2015) also reported a 100% (n=919) validation of variants identified through targeted NGS. It was concluded that validation of SNVs with a Q score of ≥20 and a high coverage of ≥100× could be unnecessarily redundant (Baudhuin *et al*., 2015). The two SNVs identified in this study had sequencing coverage of 166× and 190× (See Table 3.3, page 39). Additionally, Strom *et al*. (2014), reported a 99.090% (n=109 of 110) validation of whole exome sequencing variants and Beck *et al*. (2016), reported a 99.965% (n=5658 of 5660) Sanger sequencing validation rate. Page 101, para 2-4

While the findings of all these studies suggest that Sanger validation of NGS detected variants could be unnecessary redundant, the present study was carried out in a research setting and as standard procedure of our laboratory, all putative disease-causing variants will be validated by our Diagnostic laboratory before communicated back to patients or publishing of any study results.

2. Moreover, to have not done co-segregation analysis in the 19 family members is another major omission, and again a solid scientific justification (time constraints are not appropriate) should be provided as to why this was not done for this project. It is acknowledged that segregation analysis can provide more insight and support as to the pathogenicity, recurrence risk and potential personal implication of selected variants. However, segregation analysis was not an objective of this study and as such it was not budgeted for. While the utility of this analysis is appreciated and is therefore recommended in Future Studies, it was beyond the scope of this MSc project. Before feedback to patients, segregation analysis will hopefully be undertaken by the Diagnostic laboratory.

3. Permission needs to be obtained from journals or authors for the use of their previously published figures or tables in the dissertation. This permission must be obtained in writing and this should be documented in the dissertation. All figures and tables obtained from journals and books have been removed from the dissertation as written permission to reproduce them could not be obtained in the time allowed for these corrections. I did engage with Mrs Denise Nicholson from the Wits Scholarly Communications and Copyright Services Office on this issue. She suggested that reproduction and acknowledgement of figures "should be allowed under fair dealing in Section 12 of our Copyright Act, but because (a dissertation) goes onto the Wiredspace Open Access website after graduation, and our current law doesn't address digital issues, it is advisable to get permission at this stage". With this advice, and considering permission from publishers had not been obtained, the figures and tables were removed.

B. Minor Comments

4. Abstract: Page iv, Paragraph 2, in the sentence starting, 'Of the 15 patients recruited,' the numbers add up to 16. The number of patients recruited has been corrected.
5. Page iv, third paragraph, spelling of 'prioritise' needs to be corrected. The spelling has been corrected.
6. Page v, first paragraph, VUS needs to be defined. VUS is defined as variant of unknown significance.

Introduction

7. Page 6, first paragraph. It is stated that the prevalence for TCS is 1 in 10,000-50,000 'people' but on page 4 it states that the prevalence is 1 in 10,000-50,000 'live births'. Surely, this is not the same statistic? The prevalence for TCS has been corrected to 1 in 10, 000-50,000 live births in both statements. Page 2, para 2 and page 4, para 1
8. Page 8, first paragraph, last line. *POLR1C* needs to be italicised. *POLR1C* is now italicised, Page 5, para 1
9. Page 11, 2nd paragraph. Although not wrong, the sentence 'The author recommends ...' sound rather awkward, rather consider rephrasing to 'It is recommended ...'. The same issue is on page 39, with 'The candidate believes a Is it not better to state 'It is believed that ...' The two sentences have been rephrased. Page 9, para 1 and Page 35, para 1
10. Page 12, 2nd paragraph, the sentence starting, 'Figure 1.8 below shows a nine-year old ...' should be Figure 1.7. Figure 1.7 has been removed from the dissertation as written permission to reproduce was not obtained.
11. Page 14, Figure 1.8 is not referred to. Figure 1.8 is now Figure 1.3 and has been referred correctly. Page 11
12. Page 16, Figure 1.9 is not referred to. Figure 1.9 has been removed from the dissertation as written permission to reproduce was not obtained.
13. Page 17, the last sentence starting 'In these instances ...' appears to be incomplete, '..., suggesting genetic ' The sentence is completed. Page 13, para 1
14. Page 18, Figure 1.10 is not referred to. Figure 1.10 is now Figure 1.4 and has been correctly referred to. Page 14

15. Page 19, third paragraph. The acronym NGS has already been defined on page 5. The Definition has been removed, Page 17, para 2
16. Page 21, Table 1.4. Please define FDs and TCS in the figure legend. FDs and TCS are now defined. Page 18, Table 1.4
17. Page 21, The table heading 'genetic knowledge' is a bit strange. Is there not a better term? The term has been removed and the table is now titled "Brief review on the three facial dysostoses, TCS, MS and NS". Page 18, Table 1.4
18. Page 22, Under Objectives, 16 patients are mentioned, this should be changed to 15. The objective of the study was to recruit 16 participants; however, because of the rare nature of the disorders studied, we only managed to recruit 15 participants. Page 32, para 1

Materials and methods

19. Page 34, the text on this page needs to be in portrait format. The text is now in portrait orientation. Page 31
20. Page 35, I have major objections to this figure. The gel image is not well explained and appears to be an afterthought in the Methods section. Where are the negative controls? The bands on the ladder and the PCR fragments need to be labelled with their size. Also, it needs to be specified which variant is in which lane. Finally, should this figure not be part of Results rather than Methods? The gel image shows successful optimisation of PCRs using the seven sets of primers designed for Sanger validation. The sizes of the ladder and PCR fragments are now given and the figure is included in the Results section. Page 89, figure 3.29

Results

21. Page 37, Table 3.1 the last column labelled 'Relationship to the patient', the wording needs to be consistent here e.g. 'Mother, father and a sibling' and 'Both parents and one sibling' are the same thing. Corrected, 'Mother, father and a sibling' is used consistently. Page 33, Table 3.1
22. Page 42, | may have missed it but it does not appear that 'IGV' was defined. Integrative genomic viewer (IGV) is now defined. Page 38, para 1

23. Page 42, for the sentence, 'However, visualisation of target regions on IGV revealed that some target regions in FRASC27 were not adequately covered ... ' would it not be useful to show this as a figure? A figure showing target regions not adequately covered is included. Page 39, figure 3.5
24. Page 45, for Table 3.4 and all subsequent tables like this in this section, it would good to add the 'name' of the variants to the table e.g. p.Ala378Gly for *POLR1C* variant. A row showing protein change of all variants has been added to all tables showing selected variants in each patient.
25. Page 50, last line, IGV < 20 should be > 20. IGV > 20 is Corrected
26. Page 53, second paragraph, remove space in word 'variant s'' Space has been removed
27. Page 54, Figure 3.7 a and b these figures (as well as all subsequent figures showing similar information) need to be enlarged to improve the readability. Figure 3.7 now Figure 3.8 (a-b) and subsequent figures showing similar information has been enlarged.
28. Page 54, Figure 3.7's legend needs to be rephrased. '...a premature stop codon at position 12 downstream' does not sound right. Also, similar legends in the dissertation e.g. Figure 3.11 need to be rephrased. Figure 3.7 now Figure 3.8's legend and similar legends in the dissertation have been rephrased.
29. Page 71, last line, introduce space between '2015)' and 'Sequencing generated ...' Space has been introduced.
30. Page 87, Table 3.19 It would be good to state in this table whether the variants are homozygous or heterozygous. Also, please check the last column labelled protein change, some of the information here may be incorrect e.g. the *CHD7* variant in FRASCS9 is p.Ile1104Ter here but on page 81, a different variant is mentioned (p.Ala1237Profster70). A zygosity column was added for each variant and the protein column was removed to allow for the ACMG classification column. Page 87, Table 3.19
31. Page 88, I would suggest that the labels for the variants in these figures be made bigger. Also, 'a' and 'b' labels should be added the figures. The labels have been made bigger and labels 'a' and 'b' have been added to the figure labels. Page 88-89, Figure 26-28

Discussion

32. Page 93, first paragraph, insert gap between '2000)' and 'This abnormal ...' A space has been added.
33. Page 98, third paragraph, remove gap between 5% and -10%. The gap has been removed.
34. Page 100, 'Several study' ... should be 'Several studies ...' Wording has been corrected.