Author's response to reviews

Title: Lack of significant association between mutations of KCNJ10 or FOXI1 and SLC26A4 mutations in Pendred syndrome/Enlarged Vestibular Aqueducts

Authors:

Priya Landa (pl-1@hotmail.co.uk)
Ann-Maria Differ (ann-Marie.Differ@gosh.nhs.uk)
Kaukab Rajput (kaukab.raiput@gosh.nhs.uk)
Lucy Jenkins (lucy.jenkins@gosh.nhs.uk)
Maria Bitner-Glindzicz (maria.bitner@ucl.ac.uk)

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Author's response to reviews: see over
Re: Lack of significant association between mutations of KCNJ10 or FOXI1 and SLC26A4 mutations in Pendred syndrome/Enlarged Vestibular Aqueducts.

Dear Sir

We thank the reviewers for their comments, suggestions and questions on our manuscript and respond as follows:

Reviewer 1.
First of all, thank you for your constructive comments. The reviewer has suggested that we ‘screen ethnic-matched normal hearing controls to support the opinion that variants found in FOXI1 and KCNJ10 in the present study are only polymorphisms but not disease-causing mutations’.
Respectfully, we do not necessarily agree that this will strengthen the argument that these are only polymorphisms: this is because in KCNJ10, the variant p.Arg271Cys (c.811C>T) has a frequency of 563/8037 (or 0.07) in European Americans in EVS (http://evs.gs.washington.edu/EVS/). We now state these figures in the manuscript (page 8). At this frequency there is no possibility of this variant being anything other than a polymorphism. If it were disease-causing, it alone would account for all deafness observed in the population. Even if we were to screen another 100 individuals (200 chromosomes) and did not find the variant, the fact remains that it has been found to be very common by others.

The same applies for p.Arg18Gln (c.52G>A) in KCNJ10; the frequency is 148 in 8452 European Americans (0.0175 or 1 in 57) which would make deafness due to this mutation alone, as common as GJB2 in European Americans.
We did not screen parents for either of these variants for this reason.

However as concerns the variant in FOXI1, p.Arg123Trp (c.367C>T) we agree that as there is no frequency data on this, we need to screen controls and have done so by screening 95 pan ethnic controls for FOXI1. We have added a sentence at the end of ‘Materials and Methods: Clinical and molecular evaluation of subjects’ section on page 5, stating this, and to the Results page 6.

We have added a sentence to the effect that, whilst we cannot be sure whether this is a pathogenic mutation or not, it still appears that FOXI1 is not a major contributor to digenic inheritance in this condition (Page 8).

Thank you for pointing out that reference 34 is incorrect; we have substituted the correct reference by Chen et al now.

Reviewer 2
No Points to address

Reviewer 3
We thank the reviewer for her careful consideration and helpful comments.

We did not perform MLPA analysis of SLC26A4 in these cases. As the reviewer will be aware, in the study of Rendtorff et al 2013 in which she was the senior author, following complete screening of SLC26A4 the group found no copy number changes and so these are likely to account for a very small proportion of second
mutations, if any are present. We now mention this in the manuscript (page 10, ref 39). We have previously performed MLPA analysis in a cohort of heterozygous cases and also not found any deletions and so have now omitted this from our laboratory protocol.

In response to the request for further clinical information we have now added a further Table as Additional File 1, outlining what is known of clinical details, family history, and ethnicity of our patients. Interestingly in those cases with a family history of hearing loss, the variant often does not segregate with hearing loss, excluding a dominant mutation. Our laboratory offers segregation studies for all mutations identified yet very few requests arise for this. As can be seen from the table very few families are suitable for homozygosity mapping because of consanguinity or affected sibs (see Additional File 1).

We have added all the data on thyroid function and perchlorate tests that are available. We do agree with the reviewer that in patients with positive perchlorate discharge tests who have EVA, that the detection of bi-allelic mutations is far higher. However that was not the focus of this paper, which was on patients with monoallelic SLC26A4 mutations and the possible contribution of FOXI1 and KCNJ10 to their hearing loss.

We also agree that study of the patients for SIX1 mutations is a very good idea and thank the reviewer for that suggestion. We will do this (we cannot do this within the timeframe of this response). We also apologize for not previously citing the paper by Rendtorff et al but that paper was not published when we submitted this manuscript to BMC Medical Genetics. We do now cite this paper (ref 39).

Regarding co-incidental carriership: we think this is an unlikely explanation of much of the heterozygosity in these cases. We have recently performed an audit of all analyses performed in our lab for requests for SLC26A4 screening. This shows that where testing for Pendred syndrome is requested that 66 out of a total of 538 patients tested had monoallelic mutations (ie. 1 in 8). Whilst some of these could be co-incidental carriers, this explanation could not possibly account for all of the monoallelic cases here, otherwise carriership for SLC26A4 mutations would be almost 4 times more common than for GJB2, the commonest form of inherited deafness. However we agree that there are no good studies of carrier frequency for SLC26A4 mutations but it is likely to be less than for GJB2. We discuss incidental carriership in the manuscript (Page 9).

As suggested we have amended the comment about Yang’s study in the Introduction, by saying that the group of patients studied was heterogeneous. Nevertheless we know that the reviewer is also aware that there is an excess of heterozygotes among patients referred for SLC26A4 testing, and to date no explanation has been robustly presented.

Thank you for pointing out the typographical error, which we have clarified.

Thank you.
Yours sincerely,

[Signature]

Checked and signed electronically

Professor Maria Bitner-Glindzicz, FRCP, PhD
Professor of Clinical and Molecular Genetics