Reviewer's report

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

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Reviewer: lisbeth tranebjaerg

Reviewer's report:

Review BMC-Landa p et al- Pendred-170513

The paper adds substantial data to the possible association between mutations in the KCNJ10 and FOXi1 in cases of Pendred syndrome/EVA with one SLC26A4 mutation. The study reports a very comprehensive series of patients (68 in total) in whom the KCNJ10 and FOXi1 gene was sequenced, only with identification of fairly frequent polymorphisms. The study confirms previous smaller reports with similar results.

In general, the paper is well written with clear presentation and good relevant discussion. There are a number of questions not dealt with in the paper, however.

It is not reported whether MLPA analysis of the SLC26A4 gene was done? Furthermore, there are no data on the parents or other family members to illustrate whether there might be dominant inheritance of the SLC26A4 mutations identified. Are there parents or sibs with features overlapping with Pendred syndrome who also had the mutation? The nationality of the patients are not reported. It seems to be a nationwide UK study where a considerable proportion originate from Middle East countries. The spectrum of genetics could easily be different. And the possibility of a fraction of the families been eligible for supplementary studies (i.e. homozygosity by descent) because of multiplex affected sibs, and/or consanguineous parents are not described. The clinical features are not elaborated upon. To which degree did the authors have information about the degree and audiological pattern of hearing impairment, thyroid parameters, CT/MRI scan of inner ear and iodide-perchlorate tests performed? These data are well known to influence the yield of SLC26A4 mutations which have been low in many reports where only few patients were perchlorate tests whereas the fraction has been very high in a recent report with a high proportion of patients tested in that way (Rendtorff N et al, 2013). This paper also investigated 11 patients heterozygous for SLC26A4 mutations with identical negative findings regarding KCNJ10 and FOXi1. This paper also studied SIX1 by sequencing and identified one mutation positive case. SIX1 mutated cases have many clinical similarities to Pendred patients and it would have been good to have similar studies in the 68 patients. Surprisingly, this paper is not quoted.

Another unresolved possible explanation for the heterozygous sate of SLC26A4
mutation in the 68 patients is coincidental carrierrship and the population carrier frequency has not been studied in this paper nor reported from other countries. In the introduction it is stated that Yang et al (AJHG, 2007) found that a cohort of patients with classical symptoms of Pendred had only one SLC26A4 mutation. The molecular results in this and similar papers must be discussed in the frame of a very low percentage going through perchlorate testing. Once that test is added to the selection criteria for Pendred syndrome the percentage goes way up (Rendtorff et al, 2013).

All in all, this is an important contribution to the ongoing efforts to find out if unidentified regulatory parts of the SLC26A4 gene or neighbouring regions play a major role or whether other genes must be searched for by exome sequencing in clinically thoroughly studied Pendred –like patients.

A small typographic error occurs: in paragraph “genetic analyses, line 2, it says “where” where it should be “were”.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests’