Reviewer's report

Title: Frequency of Fabry disease in male and female haemodialysis patients in Spain

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Reviewer: Poh San Lai

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Major Compulsory Revisions

Summary:
Fabry disease is an X-linked lysosomal storage disease caused by a deficiency of alpha-galactosidase A (#-Gal A) enzyme. This gene responsible for this disorder spans 12 kb with seven exons, and more than 430 mutations have been described. Missense mutations represent the most common genetic defect in patients, correlating with residual enzyme activity of between 2-25%.

This paper reports the prevalence of Fabry disease among patients undergoing haemodialysis at 8 hospitals (including 17 haemodialysis centres) across Spain. It provides useful information for the Spanish population, the type of mutations present and the diagnostic and genetic counseling strategies for screening Fabry among the population. A total of 911 haemodialysis patients was screened and 155 samples had #-Gal A activity below the pre-defined minimum control mean. Genotyping showed two different novel mutations (in a male patient and a female patient); one previously reported mutation, R118C (in two male and two female patients) and a “pseudo-deficiency” allele, D313Y (in one male and one female patient).

Suggested revisions:
However, some improvements could be made to the paper and the following issues should be addressed:

1. Use of proper mutation nomenclature in both the text and Table 1 (genotype, nucleotide and amino acid substitution). Please refer to http://www.hgvs.org/mutnomen/ for guidelines for describing mutations at DNA, RNA and protein levels. Note that the consequences of mutations are considered as predicted unless they have been experimentally verified at the protein level.

2. Patient selection: In this study, the patients were recruited from dialysis centers and investigated for #-Gal A biochemical activity and mutations. It was stated that patients were screened for Fabry disease. The selection and inclusion criteria for the subjects should be clearly described in the Methodology. For example, was the diagnosis of Fabry based on clinical signs and symptoms, CT scans, etc.? Were patients without biopsy-proven renal diagnosis selected? Was diabetes an exclusion criteria?
3. Lack of consistency in the numbers reported and how the data is presented makes the results very confusing. The Abstract states that “GLA alterations were found in seven unrelated patients ….” In the Discussion, the authors describe “…In the present study here, we found eight individuals with GLA alterations, six with Fabry disease causal mutations and two presenting the “pseudo-allele”… In the Conclusion, it was stated that “… we identified five unrelated patients (three males and two females) with Fabry disease ..”

4. Can further evidence be provided that the two novel insertion and deletion mutations is disease-causing other than the fact that they are not found in 120 X chromosomes (how many individuals?) screened?

5. The full terminology for DBS should be written as when it first appears in the manuscript.

6. In the Conclusion, the authors claimed that “one of the mutation was present in 60% of patients, pointing out the existence of a common mutation in Fabry disease”. This may be a little to strong a point to make due to the small numbers involved, ie. with only four patients carrying the R118C mutation?

7. How would authors address the possibility that alternatively spliced variants may be present in some patients found to be negative for genomic mutations in their study? Note that Filoni et al 2008 and Migani & Morrone 2009 recommended for analysis at transcript level in patients where standard protocols fail to identify DNA mutation?

8. Table 1 has incomplete phenotype data. For meaningful interpretation of results and correlation with the genotype and biochemical data, clinical data should be provided for all the patients shown.

9. In Table 1, there is note for patient 2 stating that sister is diagnosed with Fabry, patient 5 has two affected daughters, etc. Were these patients included in this study? It would be helpful to put an additional column to indicate family history (FH), and if the two cases are related in the study, to put them together (Patients 4 and 7). This will help improve interpretation of the data presented in Table 1.

10. The authors mentioned that the overall prevalence of Fabry among renal patients in their current study (about 1 in 182 or 0.55%) was low in comparison to the study of Nakao et al 2003 (1 in 87 or 1.17%) but higher than the findings of Ichinose et al 2005 (1 in 450 or 0.22%) and Merta et al 2007 (1 in 621 or 0.16%). For population comparisons, data from a more exhaustive literature search could be included from different countries, eg. reports of incidence among renal patients of 0.22% in Netherlands (Linthorst et al 2003), 0.16% in Austria (Kotanko et al 2004), 0.3% in Belgium (Terryn et al 2008).

Level of interest: An article whose findings are important to those with closely
related research interests

**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.