Title: CYP1B1 mutation studies in patients with primary congenital glaucoma from Saudi Arabia, and identify patients with possible novel mutations in new glaucoma genes

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Reviewer: Roser Gonzalez-Duarte

Reviewer's report:

Mutations in the cytochrome P450 1B1 (CYP1B1) gene are a frequent cause of primary congenital glaucoma (PCG), a common eye disorder with an impact to the public health. Currently, the diagnosis of PCG is based on clinical findings and confirmed by genetic diagnosis. Besides CYP1B1 another gene, LTBP2 (latent-transforming growth factor beta-binding protein 2), has been linked to PCG, and several risk factors have also been described. The main aim of the work CYP1B1 mutation studies in patients with primary congenital glaucoma from Saudi Arabia, and identify patients with possible novel mutations in new glaucoma genes by Badeed OM et al. is to evaluate the contribution of exons 2 and 3 of CYP1B1 to PCG in a cohort of 34 Saudi Arabia patients (18 familial and 16 non-familial cases). From direct mutational screening (Sanger sequencing), they report three already known pathogenic variants, p.G61E, p.R469T and p.E229K, the latter not reported before in the Saudi PCG population. Concerning prevalences, the p.G61E pathogenic variant was found, in all but one case in homozygous condition, in 17/27 (63%); p.E299K heterozygous in 1/27 (3.7%) and p.R469W homozygous 4/27 (14.8%) of affected patients. The rest of the cases cases remained unsolved, 7/34 (20.6%).

Major points.

1.- The data presented adds valuable information to the genetic bases of PCG in Saudi Arabia. However, I recommend the text is revised focusing on a systematic presentation of the data. The title has to be shortened and rephrased to reflect the contents of the work. Gene abnormalities (Table 2) should be substituted by mutations.

2.- The authors report that 23 PCG affected were native Saudi (91%) and 11 non-native Saudi (54.5%) patients were positive for CYP1B1 pathogenic variants. Other authors have undertaken systematic haplotype analyses with intragenic CYP1B1 markers revealing a remarkable association between reported mutations and different haplotypes in several populations (Chavarria-Soley G. 2006, and many others). This aspect has not been addressed in the present work although it is the main issue in the discussion. Indeed, haplotype analysis would greatly improve the quality of the work as it would help to evaluate consanguinity, population substructure and founder effects for the CYP1B1 mutations in both cohorts. Besides, these data would allow comparisons of mutation-haplotype
associations in Saudi Arabia with other populations.

3.- In Table 3, the comparison between the severity of the disease of native and non-native Saudi Arabia patients should specify the subtype of congenital glaucoma, the mutation found and the age of the patients to address the correlation between CYP1B1 mutations and the severity of the disease (e.g. Hollander DA et al. 2006). Merging Tables 1, 2 and 3 would help the reader without loosing any relevant information.

4.- Given that all the mutations and polymorphisms have already been reported by several groups (including Abu-Amero et al. 2011 on Saudi families), tables 4 and 5 should be omitted. Instead, one table with the genotype assignments, detailed compound heterozygosis and the unsolved cases should be presented.

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

**Quality of written English:** Needs some language corrections before being published

**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