Author's response to reviews

Title: CYP1B1 mutations in patients with primary congenital glaucoma from Saudi Arabia

Authors:

Osama M Badeeb (obadeeb@yahoo.com)
Shazia Micheal (shazia.mickeal@radboudumc.nl)
Robert K Koenekoop (robert.koenekoop@mcgill.ca)
Anneke I den Hollander (anneke.denhollander@radboudumc.nl)
Manal T Hedrawi (manaley@hotmail.com)

Version: 2 Date: 7 August 2014

Author's response to reviews: see over
Dear Prof Sands,

Thank you very much for considering the publication of our article: *CYP1B1* mutation studies in patients with primary congenital glaucoma from Saudi Arabia identify patients with possible novel mutations in new glaucoma genes. We addressed all the reviewer comments in our revised manuscript, and here our responses in red print.

**To Prof: Alexander Bialasiewicz comments:**

1) PCG should be detailed regarding genetic work-up and compared to relevant literature (Khan AO1, Aldahmesh MA, Mohamed JY, Hijazi H, Alkuraya FSJ AAPOS. 2012 Dec;16(6):571-2. CYP1B1 analysis of unilateral primary newborn glaucoma in Saudi children.). We have now done this and it is addressed on background page (4) lines (73-75) and discussion page (11, 12) lines (208- 222).

2) In the discussion the Western part of the Kingdom is stressed regarding genetic ethnic diversity, however, the Eastern provinces with their significant Iranian influence is not mentioned. We have now done this, and we addressed these points on the background page (4) lines (65-67, 73-75) and discussion page (10) lines (181- 184). Non-native Saudi PCG patients were excluded from all previous gene studies, as these previous studies only conducted testing on native patients from various tribes, and regions of SA [4-8]. To the best of our knowledge our study is the only gene study in SA, which address the effect of ethnicity on gene anomalies in PCG. No studies were conducted in the Eastern region of Saudi, so we can’t address the significant of Iranian influence on PCG gene anomalies.

3) For completeness, the Omani El-Gayar S1, Ganesh A, Chavarria-Soley G, Al-Zuhaibi S, Al-Mjeni R, Raeburn S, Bialasiewicz AA.Mol Vis. 2009 Jul 8;15:1325-31. Molecular analysis of CYP1B1 in Omani patients with primary congenital glaucoma: a pilot study.) and the Kuwaiti study (Alfadhli S1, Behbehani A, Elshafey A, Abdelmoaty S, Al-Awadi S. Am J Ophthalmol. 2006 Mar;141(3):512-Molecular and clinical evaluation of primary congenital glaucoma in Kuwait.) should be included/discussed briefly. We have now added this and is addressed in discussion page (11) lines (200-202, 206-207)

**To Prof Roser Gonzalez-Duarte comments:**

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.

We have changed abnormalities to mutations.
We have now shortened the title to: CYP1B1 mutations in patients with primary congenital glaucoma from Saudi Arabia.

We have merged the tables as suggested by the reviewer, to become 3 instead of 5.

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.

This point is now addressed in the discussion, page (11, 12), lines 208-222.

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).

We have done this. This table changed to table (2), which now correlates between the severity of PCG and the CYP1B1 mutations. This correlation, mentioned in the results page (8) lines (136-138) and discussed in details at page (12) lines (223-231).

Regarding the age of onset of PCG of all our patients; it was at birth and all our patients had PCG and no other types of congenital glaucoma. We have mentioned that in the results, page (7), line (122).

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.

Tables (4, 5) changed to table (3)

Thank you again for considering our manuscript and a special thanks to the reviewers for their time and valued comments.

NB: All changes were highlighted in the manuscript by red print.

Sincerely yours
Osama Badeeb
Principal author
7/8/2014