Author's response to reviews

Title: Sequence variations in the human PAX6 gene with aniridia in south Indian population

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PDF covering letter
Dear Dr. Matt Hodgkinson

Editorial Assistant, BMC Medical Genetics

Thank you for sending us the reviewers’s comments on our manuscript entitled “Sequence variations in the human PAX6 gene with Aniridia in South Indian Population” (1358717106269565). We have substantially revised and rewritten our manuscript as per the reviewer’s comments. We have addressed the reviewer’s comments in detail and our responses are set out below.

We look forward to hearing from you soon.

Yours sincerely,

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**First Reviewer’s Report:**
Minor Essential Revisions
In the Result session, the gene accession number for use of nucleotide numbering was not provided.

The requested changes have been made to the results section.

In the last paragraph on page 7: Did the proband 16-1 show only lens dislocation or also systemic anomalies matched with Marfan syndrome?

This patient showed Marfan syndrome with subluxated lens and secondary glaucoma. Since the Marfan syndrome is rarely associated with aniridia, and thus it has to be consider hardly. The wrist sign and the thumb sign were positive, cardiac examination revealed mitral valve prolapse.
Second Reviewer’s Report

In the background section some precision have to be brought out. The prevalence of the PAX6 mutations is low, between 1/50,000 and 1/100,000, and they often cause blindness through a spectrum of ocular manifestations among which aniridia and the most probably foveal hypoplasia are the major signs.

The requested changes have been made

The results section is particularly hard to follow as the mutations description deals together with nucleotides, codons and aminoacids. It would benefit to comply with the mutation nomenclature as described in (Hum Mutat 2000; 15 :7-12, Hum Genet 2001 ; 109 : 121-124 ) (e.g. the five bases duplication in codon 118 should be mentionned as a cXXXXins5 or cXXXXinsAATCC, etc.).

We have rewritten the results section to understand clear about the nomenclature of the sequence variation.

We have removed the predicted protein sequence (fig 4 and 5). In fact it is very unlikely that nonsense mutations result in truncated proteins. The mutation is unlikely to produce a protein, because the mutant mRNA would be degraded by nonsense-mediated decay’. We have inserted the reference of Byers PH for the appropriate points.

The simultaneous presence of two sequence variants in proband 27-1 as to be further precised. How did the authors detect its presence in cis ? Was it always present on the sequenced cloned PCR product ? Did they do their best to withdraw a PCR induced nucleotidic change ? Was it a de novo event in this consanguineous family, was it present in asymptomatic members. What is the c.1239A>G presumptive impact on the gene function ?

We did the PCR again (starting from genomic DNA) for sequencing which gave superimposed signals from the deletion mutation, In addition we have sequenced more than one clone to show that both changes are always present. This would prove that the changes were really present in the
patient, and not generated by Taq error. The consequence of this change is unknown. It is likely that the other mutation (c.1201delA) is the one responsible for aniridia.

As for proband 10-1 the authors cannot affirm as it that the absence of the nonsense mutation of all unaffected family members confirm the sporadic nature of the disease. In fact this is only in accordance with assuming the complete penetrance of PAX6 nonsense mutations and the absence of a germinal mosaicism in one of the parents.

We have changed the ‘inconsistent sporadic nature’ in the text but we have sequenced the parents of the proband 10-1 which showed normal chromatogram signals

Why is there a separate section for « heterozygous non sense mutations » in the results section ? Reporting R40X or W156X should arise some questions on the « recurent » nature of these mutations. The author should have briefly discuss on the meaning of this findings in different populations : is it relevant to mutation hot spots or founder effects ?

We have changed the requested section and now we have indicated clearly in the discussion section.

The discussion on the nature of the IVS9-12C>T is inapropriate as the C>T nucleotidic change in this position in splicing acceptor sites does not deeply affect the splice in numerous genes and has still been reported as an innocent variant in the PAX6 gene. Do the authors analysed the unaffected proband’s relatives ?

Yes, we have analysed but we have not find any difference both in SSCP as well as in automated sequencing reactions.

PAX6 mutant are now well known to display panocular malformations as well as some extraocular involvement. Most of the signs reported by the authors have been previously described but I wonder what kind of corelation they wanted to draw when they described a Marfan’s phenotype in proband 16-1 ? The presence of a nonsense heterozygous mutation in the linker domain encoding part of the gene appears really convincing as the cause of the aniridia phenotype. The Marfan diagnosis would need to meet some other major criterion than the lens ectopia, are they present ? In addition the high incidence of Marfan’s syndrome (Fibrilin) new mutations in the population has to be considered.
This patient showed Marfan syndrome with subluxated lens and secondary glaucoma. Since the Marfan syndrome is rarely associated with aniridia, and thus it has to be consider hardly. The wrist sign and the thumb sign were positive, cardiac examination revealed mitral valve prolapse.

Did the 10-1 proband ptosis occur before surgery? The paper of Malandrini et al. dealing with the presence of a ptosis (ref 18) may in fact report a fortuitous association as the presence of a mental retardation in heterozygous PAX6 mutations is by no means proven.

**Ptosis occurred before surgery hence the association with aniridia is common.**

In the discussion section, the term development of the iris (p8 l4) has to be replaced by development of the eye.

**The requested changes have been made.**

The c.1239 A>G has to be introduced in the result section before being discussed here.

**The changes have been made.**

The discussion of the IVS9 polymorphism is to be dropped out. The conclusion that a mutation should lay in another part of the gene in patient 18-1 should be reapraised after using a more efficient screening procedure than SSCP.

**We have précised the explanation of the polymorphism. As an alternative to SSCP we have performed the heteroduplex analysis stained by ethidium bromide, which showed no difference in all the exons for the 18-1 pedigree, if the reviewer wants to see we will send the picture,**

To my own opinion the sequencing of both genomic DNA and mRNA would be more efficient and less time and energy consuming than cloning each amplicon of the gene.

**We performed the amplicon cloning because the genomic PCR sequencing failed for two times.**
The abstract has to be deeply revised as it has no structure and make no sense sometimes speaking of « variable phenotypes » or « well-characterized aniridia » of six defined aniridia « families » constituted of « sporadic » cases. In addition its conclusion does not bring nothing as it is well known that PAX6 cause pathological aniridia.

On the opposite a discussion of the genetic homogeneity of aniridia would have been more interesting as all but one cases have been demonstrated to have a PAX6 mutation. Such a detection level is unusual when SSCA is used as the primary screening procedure and we would have appreciated to know further on the precise ethnical background of the population and the total number of families tested.

We have rewritten the abstract section as per your comment.

Tables and figures: In table 2 the predicted aminoacid structure is purely speculative and unneeded in this mutation report. The sequencing method should be mentionned in figure 3 instead of table 2 as the presence of both hemizygous and heterozygous mutation on electropherograms is confusing.

We have modified the text in the figure 3 to understand clear about the changes.

In figure 2, showing the pedigrees does not bring nothing to the reader as all proband are sporadic cases and testing the number of unaffected relative (if tested) can be mentionned in text. The SSCA profiles are unnecessary and figure 2 and 4 could be deleted.

SSCP is said to be a common method for mutation analysis with 70-80% sensitivity. We were able to detect all reported changes by this method, so we would like to focus this method also as an effective one in PAX6.

Figure 5 should be omitted and replaced by a clear description of the mutations in teh results section.

This has been done as requested.