Reviewer's report

Title: Sequence variations in the human PAX6 gene with Aniridia in South Indian population

Version: 1 Date: 17 February 2004

Reviewer: Patrick CALVAS

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please find below the major revisions I asked the authors before being able to judge their report which could be interesting if deeply revised. The paper is of a limited interest as it and the revision might include new experiments (mutation testing).

The report of PAX6 three novel mutations in the South indian population by Neethirajan et al. is an interesting fact. However, the paper is confused and should be exhaustively rewritten and revised before publication.

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 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 mentioned as a cXXXXins5 or cXXXXinsAATCC, etc.).

The presumptive impact on protein sequence should be given cautiously as, all the studies dealing with mRNA analyses seemed to conclude to the absence of transcripts from nonsense mutations bearing alleles.

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 ?

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.

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 « recurrent » 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?

The discussion on the nature of the IVS9-12C>T is inappropriate 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?

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.

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.

In the discussion section, the term development of the iris (p8 l4) has to be replaced by development of the eye. The c.1329 A>G has to be introduced in teh result section before being discussed here. The discussion of the IVS9 polymorphim is to be dropped out. The conclusion that a mutation should lay in another part of the gene in patient 18-1 should be reappraised after using a more efficient screening procedure than SSCP.

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.

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.

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.
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.
Figure 5 should be omitted and replaced by a clear description of the mutations in teh results section.The report of PAX6 three novel mutations in the South indian population by Neethirajan et al. is an interesting fact. However, the paper is confused and should be exhaustively rewritten and revised before publication.

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What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest