Author's response to reviews

Title: Identification of a novel homozygous nonsense mutation in EYS in a Chinese family with autosomal recessive retinitis pigmentosa

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Version: 3 Date: 11 May 2010

Author's response to reviews: see over
Author’s response to reviews

Title: Identification of a novel homozygous nonsense mutation in *EYS* in a Chinese family with autosomal recessive retinitis pigmentosa

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Version: 2  Date: 10 May 2010

Author’s response to reviews: see over
Reviewer’s report

**Title:** Identification of a novel homozygous nonsense mutation in EYS in a Chinese family with autosomal recessive retinitis pigmentosa

**Version:** 1  **Date:** 5 April 2010

**Reviewer:** Muhammad Ansar

**Reviewer’s report:**

1. In abstract, conclusion section, line five authors should replace studies with “study”.
   - Corrected. Thanks.

2. Background section, second paragraph, end of line four authors should replace causes with “cause”.
   - Corrected.

3. Background section, third paragraph, end of line 4. Authors should delete the entire sentence starting from “Though forty three candidate genes---- arRP (11). As it is not important to mention this once gene is identified.
   - This sentence has been deleted in the revision.

4. Background section, fourth paragraph, third line one should be replaced with “a” and authors should delete text “in the newly identified RP25 disease causing” in the fifth line. These words are repeated many times in the entire manuscript.
   - These two changes have been done in the revised MS.

5. Method section, first paragraph, authors should mention manufacturer details of DNA isolation kit.
   - Yes, we have added manufacturer details of DNA isolation kit as the reviewer suggested.

6. Result section, last paragraph, fourth line it should be “glutamic” instead of glycine.
   - Thanks, the error has been corrected.

7. In figure legends, figure 1, authors mentioned rs4710292 and rs4710434, which
were not mentioned in the entire manuscript. Even these SNPs are not shown in figure 1.

- Sorry, we submitted a wrong figure 1 by mistake. In order to get definite haplotype map, we had genotyped six SNPs (rs4710437 (sorry, not rs4710434), rs1057530, rs4710457, rs66462731, rs10944813, rs4710292), which are close to EYS. This data has already added to line 10-11 in the second paragraph of method section, and the last figure 1 edition is enclosed in the revision.

8. Additionally in haplotype authors have depicted markers which are quite apart from each other. Like marker D6S1610, which is homozygous in three affected individuals is 27 cM away from EYS gene. This point requires clarification as these affected individuals are heterozygous at marker D6S257, which is close to EYS as compared to earlier mentioned marker. The data presented in the haplotype by the authors does not support linkage to EYS gene. Authors should remove haplotype or genotype more markers to get clear linked haplotype.

- The reviewer is right. We have presented the new edition figure 1 in the revision.

9. Finally authors should discuss relationship (if any) between the site of EYS truncation and severity of the phenotype, to enable phenotypic comparison with current study.

- It is an excellent suggestion to discuss the relationship between the EYS truncation and clinical severity of RP. We discussed the RP combined cataract symptoms in one of our patient. The similar symptoms were presented in patients with p.T3156X carrier, described by Collin et al. However, we are short of enough data to summarize the relationship between EYS truncation and the phenotype of RP.
Reviewer’s report

Title: Identification of a novel homozygous nonsense mutation in EYS in a Chinese family with autosomal recessive retinitis pigmentosa

Version: 1 Date: 23 March 2010

Reviewer: Rob WJ Collin

Reviewer’s report:

Huang et al. describe the identification of the first nonsense mutation in EYS causative for arRP in the Chinese population. The identification and causality of the mutation is clear-cut and the clinical data are well-presented. However, a number of issues need to be addressed that will improve the quality of the paper. Major compulsory revisions:

1) The mutation is present in a homozygous manner in affected family members born from a consanguineous marriage. The haplotype analysis shown in figure one however suggests that the mutation is present on two different alleles (only one marker is homozygous in all affected members). Either the homozygous region flanking the mutation is relatively small, or the mutation occurs on two different haplotypes which might suggest that this mutation might be more frequently present in the Chinese population. The distance between the markers flanking the EYS gene (D6S257 and D6S460) is 24 Mb of genomic DNA. The authors should analyze more markers in close proximity of the EYS gene, to see whether the mutation is present on the same or on two different haplotypes.

• Figure 1 that we submitted in the manuscript isn’t the correct one. We are sorry for the mistake. Actually, we had genotyped six SNPs (rs4710437 (sorry, not rs4710434), rs1057530, rs4710457, rs66462731, rs10944813, rs4710292), which are close to EYS. This experiment has already added to line 10-11 in the second paragraph of method section, and the last edition figure 1 was enclosed in the revision.

2) Based on studies performed by others, the frequency of EYS mutations in recessive RP patients is relatively high (5-10%). Here, the authors describe a single mutation in a Chinese family. Do the authors have DNA of other Chinese RP patients? If so, have they been analyzed (either by linkage or sequence analysis) for mutation in EYS? If not, I think the authors should test these for mutations in the EYS gene, at least starting with the mutation that was identified here. Data about the prevalence of EYS mutations in the Chinese RP population
would definitely strengthen the manuscript.

- Thanks for the excellent suggestion. We are planning to collect more adRP pedigrees and patient. If we recruit enough patients more fund, we will perform the investigations.

3) The authors claim to be the third group (after the two initial papers identifying the EYS gene) that present mutations in EYS. However, a paper just appeared in IOVS describing several novel mutations in EYS by Abd El-Aziz et al. (Manuscript iovs.09-5109, published on March 17, 2010). Please refer to this paper.

- When we submitted the manuscript, only two papers about EYS associated with arRP were available. We though it was true that we were the third groups that present mutations in EYS when we submitted the MS. However, just as the reviewer point out, three papers about EYS mutations (including Abd El-Aziz et al.) were published very recently.
- As one reviewer suggested, the three papers have been referred in the Note Added in Proof section in the revision.

Minor essential revisions:
1) Add page numbers.
- Thanks. We have added the page numbers in the revision.

2) Abstract, conclusions: EYS encodes the orthologue and not the homologue of Drosophila spacemaker. Please correct.
- Corrected.

3) Background section, first page: RP; OMIN should say OMIM
- Corrected.

4) Background section, second page: ‘…which is predicted to be a 3,145 amino acid’. The protein has 3,165 amino acids. Please correct.
- The 3,165 amino acids of EYS protein has been used in whole revised manuscript (Genbank accession No: FM209056).
5) Background section, second page: ‘…from Spanish and Dutch…’ Please add the word ‘origin’.

- Thanks, and revised.

6) Results, second page: ‘To identify the disease causing EYS mutation..’. Please remove EYS.

- Corrected.

7) Discussion, first page: ‘EYS is a multi-domain protein with 3,1645 amino acids and containing…’ Please remove ‘with 3,1645 amino acids and’ as this has been stated in the previous sentence.

- The mentioned sentence has already revised in the revision.

8) Discussion, second page: Upon mentioning the previously identified mutations, remove the family names. This has no additive value at all.

- Revised.

9) Discussion, second page: Be straight in mutation nomenclature throughout the manuscript, use either single-character or three-character amino acid codes, but not both.

- Thanks, the p.E1836X is used through the whole manuscript now as suggested.

10) Discussion, second page: Elaborate on the recently published paper by Abd El-Aziz et al., as they also identify missense mutations.

- We have been referred the very recently published papers about EYS mutations (including Abd El-Aziz et al.) in the Note Added in Proof section of the revision.

- 11) Discussion, third page: …from two different families with mutation p.Pro3156X…’ should state ‘with mutation p.Tyr3156X’.

- The error was corrected in the revision.

12) Discussion, third page: ‘…in the cataractogenesis in addition to the retinas.’ Replace retinas by retinal dystrophy.

- Corrected.
13) Figure legends, figure 1: There is no description of the genomic positions of the markers in the legend. In addition, add which genome browser working draft was used to extract positions.

- The genomic positions of the markers are showed in the figure 1 in the revision.

14) Figure legends, figure 2: Briefly describe the findings of the ERG recordings in the legend.

- The figure legend has been revised.

15) Figure legends, figure 3: I think exon 2 is a typo, and should state a different exon.

- The error has been corrected in the revision.

Discretionary revisions:
1) Figure 3: Adding the amino acids encoded by the triplets of nucleotides above the sequence chromatogram would improve the figure.

- We will greatly appreciate the reviewer for all of the kind comments. The figure has been improved in the revision.
Reviewer’s report

Title: Identification of a novel homozygous nonsense mutation in EYS in a Chinese family with autosomal recessive retinitis pigmentosa

Version: 1 Date: 30 March 2010

Reviewer: Guillermo Antinolo

Reviewer’s report:

In the present article, Huang et al. report a new truncating mutation in Eyes Shut Homologue (EYS) in a consanguineous Chinese family affected by severe autosomal recessive retinitis pigmentosa (arRP). Firstly, they employ linkage and haplotype analysis for the identification of EYS as the causal gene following sequence analysis to identify the disease-causing variation. The candidature of the identified mutation as the genetic event triggering the pathogenic mechanism of such retinal dystrophy phenotype is supported by the truncating nature of the change, which would lead to the translation of EYS into a significantly shorter protein. This, together with the family segregation of the variation consistently with the consanguinity of the family, and its absence in 200 control individuals, makes the authors to conclude that p.E1836X is the pathogenic variation that triggers the disease in this family.

The authors thank the reviewer for the positive comments.

Minor Essential Revisions
1. The authors present their results as the first independent confirmation of the two original reports of mutations in EYS as the cause of RP, as well as the first report of a mutation identified in the Chinese population. However, during the running month, two papers have been published reporting a wider spectrum of EYS mutations in different populations, including mutations in a Chinese family. Therefore, we suggest that the authors include a ‘Note Added in Proof’ referencing these recent reports.

   • The reviewer is right. We have added the ‘Note Added in Proof’ section in the revision.

2. The manuscript is poorly written. We strongly recommend a native-English revision of the language that would greatly improve the quality and comprehension of the content.

   • We are grateful for Dr. Stephen R Archacki, for his critical reading and
improving of the manuscript.

3. Given the importance of RP phenotype characterisation when presenting results based on a single family, we suggest that Table 1 is included within the manuscript data and not as supplementary information.

   - According to the suggestion, we deleted the table in the revision. The RP characterizations of the family members have been already described in the result section. The Table 1 was deleted in the revision.

4. The authors should revise the current number of arRP loci, which is incorrect as described in the manuscript.

   - Thanks, the sentence has been changed to “About 30 genes and loci have been implicated in isolated cases of arRP to date”.

5. Regarding the prevalence of the genes implicated in arRP, they should include some references, especially of recent reviews on the subject.

   - We cited the papers (ref. 4, 5, 6) in the revision as required.

6. In page 3, line 19, the term differentiation is incorrectly used. The authors should employ other terms such as ‘classification’, and the like.

   - Done. Thanks.

7. We have found some disorder in the references numbering, for example, in page 4, line 4, Barragan et al. is reference 11 and not 10.

   - Thanks, and corrected.

8. When describing the identification of EYS and its structure in page 4, lines 6-10, the authors should reference Abd El-Aziz et al., 2008 (4).

   - Yes, the reference was added here in the section.

9. In page 6, line 14, the authors describe the visual acuity in the three affected individuals as limited to hand movements. However, in Table 1, which they refer in this line, they use this term for the description of the visual acuity only in family member II5. They should try to keep the consistency by using a different term or sentence to report the visual acuity in the manuscript text.
• Thanks for pointing out this mistake. The descriptions for the family members have been corrected in the main text.

10. We recommend that the authors include the mutation state of family member III1 in the results text, given that they are showing the corresponding chromatogram information in Figure 3.

• The heterozygous state of III1 was described in the revision.

11. There is a depiction error in Figure 1 regarding the genetic markers employed for the analysis. Whereas the figure legend describes the haplotype as conformed of SNPs (rs) and homozygous, the figure depicts microsatellite markers showing heterozygosity around EYS region.

• We are sorry for present wrong edition of the figure. The right figure 1 and figure legend has been revised in the revision.

12. In the discussion section, authors hypothesise that EYS may also play an important role in the cataractogenesis of patient II5. To our view, this conclusion is too speculative, and should be supported by additional evidence.

• Though many cases have been reported that some patients may combine cataract with retinitis pigmentosa, and we believe that the two eye diseases maybe have some common pathogenic basic. The authors agree the reviewer’s opinion. The hypothesis “EYS may also play an important role in the cataractogenesis” may be too speculative.

The article describes an interesting novel mutation in EYS as the cause of the disease in a consanguineous Chinese family affected by arRP. Interestingly, this mutation has not been found in any of the recent reports on EYS prevalence in different populations including the Chinese one. Furthermore, this finding supports the hypothesis of a global EYS involvement in the disease development of the arRP, confirming the initial linkage data in populations of different ancestral origins. Therefore, we find this manuscript appropriated to be published in BMC Medical Genetics upon addressing the previously enumerated comments as revisions.

• The authors appreciate the reviewer for the positive comments.