Author's response to reviews

Title: MRPS18CP2 alleles and DEFA3 absence as putative chromosome 8p23.1 modifiers of hearing loss due to mtDNA mutation A1555G in the 12S rRNA gene

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Author's response to reviews:

Dr. Melissa Norton
BMC Medical Genetics Editorial Office

Barcelona, 6th November 2007

Dear Editor,

Please find enclosed the revised version of the manuscript ¿MRPS18CP2 alleles and DEFA3 absence as putative chromosome 8p23.1 modifiers of hearing loss due to mtDNA mutation A1555G in the 12S rRNA gene¿ according to the helpful reviewers¿ comments. We are also including a point-by-point response indicating the changes introduced across the manuscript. We would appreciate your considering this manuscript for publication in the Journal.

As already mentioned, all authors of this research paper have directly participated in the planning, execution, or analysis of the study, and have read and fully approved the final version here submitted. The contents of this manuscript have not been copyrighted or published previously, and are not now under consideration for publication elsewhere. Finally, there are no directly related manuscripts or abstracts, published or unpublished, by any author of this paper.

We hope that you and your editorial board will provide a positive assessment of this manuscript. We look forward to hearing from you.

Sincerely,

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Response to referees

Referee #1

Minor Essential Revisions

1. Page 2, line 4, revise ¿Between¿ as ¿Among¿?

Following the reviewer¿s suggestion the term between has been changed for among.

2. Figure 3 needs major revisions: the vertical bars in Fig. 3A showing identity between MRPS18C gene and MRPS18CP2 pseudogene need adjustments to show the exact matches. In Fig 3B, the labeling of tissue names is very confusing, as the current version did not show any information regarding which lane refers to which tissue.

Figure 3 has been completely remade.

Referee #2

Major Compulsory Revisions

1. In families with the A1555G mtDNA mutation, there was a statically significant association with deafness and a polymorphism in the MRPS18CP2 pseudogene and an ¿overrepresentation¿ of DEFA3 gene absence. Nevertheless, it is not possible to conclude that these genetic variants contribute to the disease pathogenesis as implied by the title, which must be modified. Because all of the families were of Spanish origin, the genetic associations with deafness could be due to founder effects.

We agree with the reviewer in that the associations found are weak and not conclusive. Therefore the title of the manuscript has been changed for the following:

¿MRPS18CP2 alleles and DEFA3 absence as putative chromosome 8p23.1 modifiers of hearing loss due to mtDNA mutation A1555G in the 12S rRNA gene¿

In addition, it is mentioned several times in the text that the results presented here are only another hint of the possible implication of these region in the pathogenesis of deafness linked to A1555G mutation (pg 15, line 13; pg 16, line 14 and conclusions). It is also important to take into account that the present
work is the only published result showing an additional evidence of the implication of 8p23.1 region as a modifier factor, since the region was first identified by Bhykovskaya and colleagues (Am J Hum Genet. 2000 Jun;66(6):1905-10).

Regarding founder effects, they were previously studied and discarded by Torroni and colleagues (Am J Hum Genet. 1999 Nov;65(5):1349-58.). The families in our work come from the same set of families as for the work of Torroni and colleagues. Thus, although founder effects are an important point in genetic association studies, they could not account for the association found in the present work. However, to clarify this point, the following sentence has been included in the discussion section:

Although most of the analysed samples come from the same geographic area, founder effects do not account for the association found as it was previously reported (ref Torroni).

Minor Essential Revisions

1. In the Conclusions of the Abstract, Although any of the factors should be Although none of the factors.

Following the reviewer’s suggestion, the above sentence has been replaced by Although none of the factors.

2. In the Results (page 11, text line 5), expands should be spans.

The word expands has been replaced by spans, in agreement with the reviewer’s comment.

3. In Table 1, decimal points should be . not ,.

The decimal points have been corrected in all tables.

Discretionary Revisions

1. It would be interesting to know the size of the chromosome 8p23.1 modifier locus and the number of genes and pseudogenes in this region.

The detailed information on the localization of the modifier locus has not been included in the present work as it was already published when the locus was described (Am J Hum Genet. 2000 Jun;66(6):1905-10 and Genet Med. 2001 May-Jun;3(3):177-80). However, in figure 1 the marker with the highest lodscore is placed with respect to the analysed features in the region.

Referee #3

Discretionary Revisions

The present paper described a candidate nuclear modifying locus for modulating
the phenotypic variation due to the 1555 A>G mitochondrial mutation. The authors analyzed three candidate genes by means of PCR-RFLP or direct sequencing. The data obtained showed weak association with the MRPS18CP2 pseudogene and DEFA gene absence, though detailed mechanisms were uncertain. Though the authors' group have extensively studied the modifier genes, the paper unfortunately could not provide conclusive evidence that these genes are really related with the phenotypic variation of the patients with the 1555 mitochondrial mutation.

1. As the association was very weak (as the authors stated) the title of the paper is too conclusive.

We agree with the reviewer that the title is too conclusive taking into account the weak association found. Thus, the title of the manuscript has been changed for the following:

¿MRPS18CP2 alleles and DEFA3 absence as putative chromosome 8p23.1 modifiers of hearing loss due to mtDNA mutation A1555G in the 12S rRNA gene¿

2. Selection criteria for these three genes were uncertain, and should be clarified.

In accordance with the reviewer’s suggestion, the following sentence has been added to clarify the selection criteria used (pg 4, last paragraph):

¿These genes were selected after an exhaustive screening of the region looking for candidates as genetic modifiers of A1555G associated phenotype. Defensins were chosen because of their close proximity to the positive linkage region and CLDN23 and MRPS18CP2 were selected on the basis of their putative biological function.¿

In addition, selection criteria are already mentioned in pg 10, first paragraph; pg 11, last paragraph and pg 13, line 24.

3. There are problems with alignment in Fig. 3A due to the font used.

As suggested by the reviewer, the alignment on figure 3 has been remade.