Reviewer's report

Title: Molecular epidemiology of deafness in France reclassifies mutations as common polymorphisms in the GJB2 gene

Version: 1 Date: 30 December 2003

Reviewer: Ignacio del Castillo

Reviewer's report:

General
The manuscript by Roux et al. reports the results of a genetic screening for mutations in the DFNB1 locus in a cohort of 84 unrelated families with non-syndromic congenital hearing impairment. The authors also present the results of a massive screening of GJB2 mutations in newborns (several thousand subjects), which constitutes the major originality of this work. Data are sound and well controlled. Based on their epidemiological data, the authors reclassify several sequence variations in GJB2 with regards to their putative pathogenic roles.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
My only major criticism regards the conclusions concerning the pathogenic potential of the M34T allele. As posed by the authors, the pathogenicity of some GJB2 alleles remains controversial. It is commonly accepted that there is not a good genotype-phenotype correlation in the DFNB1 type of deafness, i.e. even the very common 35delG/35delG genotype can result in hearing losses ranging from mild to profound. This variability can be due to the characteristics of each of the two alleles found in a given subject, to the genetic background (modifier genes) or to the interaction of the genotype with environmental agents (e.g. noise exposure). Given that this situation represents a serious practical problem for molecular diagnosis and genetic counselling, extreme caution is needed when confirming or excluding pathogenic roles of specific alleles. Based on their epidemiological data, the authors conclude that M34T, V37I and R127H 'could no longer be considered as recessive mutations' (abstract, page 2). Furthermore, they add 'we can definitely eliminate the possibility of considering the M34T as a dominant or recessive mutation' (page 11, lines 24-25). However, in the Discussion section, the authors state that 'we still cannot rule out that the combined genotype R127H/M34T...under certain circumstances ...would participate to the phenotype' (page 12, lines 9-12). And also: '...once more we cannot rule out that the genotype M34T/V37I is not associated with deafness.' (Page 12, lines 23-24). These contradictory messages mean that the controversy on the pathogenicity of these alleles is far from being solved. I recommend that the authors rewrite this part of the discussion indicating very clearly what it is well established at present, namely i) that M34T is not a dominant mutation; ii) that it is not responsible for severe or profound deafness even in compound heterozygosity with more harmful alleles (inactivating mutations such as 35delG); iii) that it is a very common allele, at least in the Languedoc-Roussillon population. Then it should be mentioned that, in cases with recessive inheritance, M34T homozygotes and M34T compound heterozygotes might have or might have not mild to moderate hearing impairment (the authors report one of these cases, M34T/V37I, in Table 2; there are more in the literature); take also into account that some mild hearing impairments may be overlooked in neonatal clinical screenings, and that the age at onset might be also variable. The factors that are hypothetically responsible for this lack of predictability (see above) should be discussed. Indeed, what the authors' data and the accumulated evidence in previous reports really mean is that we cannot infer general rules on the pathogenicity of M34T, i.e. its effects should be examined individually in each patient. This conclusion may look a little discouraging, but it should be expected that the situation changes after identification of the postulated modifier gene(s) of this
phenotype. (The abstract should be also modified accordingly, to tone down the last sentence of the conclusion).

As regards this issue, I have one additional question. One M34T homozygote was found in the neonatal screening. Which was his/her clinical status as regards hearing? A follow-up of this case would be interesting.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) Title: It should read "Molecular epidemiology of DFNB1 deafness in France reclassifies...", because the authors have investigated the genetic epidemiology of only the DFNB1-type of deafness.

2) Abstract, Conclusion, line 3: ‘dependant’ should read ‘dependent’.

3) Page 3, paragraph 2, line 2. Since so many articles have been published regarding the contribution of DFNB1 to deafness in different countries, the choice of references 4-10 is arbitrary. It would be better to cite a comprehensive review (for instance, reference 28).

4) Page 3, last paragraph, first line. According to the latest sequencing data, the deletion encompasses 309 kb (see reference 26, which should be cited here). Please correct also page 7, line 19, accordingly.

5) Page 4, paragraph 2, line 4: insert ‘mutation’ after ‘the 35delG’.

6) Page 5, line 3: include ‘symmetrical’ (only assymetrical or unilateral hearing losses are mentioned). Also in this paragraph, didn’t the authors find any case with ambiguous (unclear) inheritance pattern? (Compare with next paragraph).

7) Page 5, paragraph 2: How many of the families were non-syndromic? Also, replace ‘moderate’ by ‘mild’ in the second line (mild is a less severe degree of hearing loss than moderate). The authors report 55 families with a single deaf child. Was the family history known in all these cases?

8) Page 5, Audiological assessment: The authors should include the criteria for the classification of the different degrees of severity, in dB intervals.

9) Page 5, last paragraph: explain the meaning of the PSDM acronym (PCR-mediated site-directed mutagenesis).

10) Page 6, line 4: delete ‘the’.

11) Page 6, Results. It is not clear what the authors mean by ‘entire non-coding GJB2 region’, since the protocol is not described in the Methods section. Have the authors checked the first exon and the whole 3’ UTR of exon 2?

12) Page 6, Results, last sentence: ‘...all patients... had bilateral congenital severe or profound NSHL...’ Not all, three patients in Table 1 are shown with moderate deafness.

13) Page 8, line 10: It should be 6,293 newborns (2,777 plus 3,516; see also Table 4).

14) Page 10, line 3: the authors report 33 sequence variations (not 32; 11+C64X+21).

15) Page 13, line 1: substitute ‘asks’ by ‘wonders’; line 13, insert ‘in degree of severity’ after ‘composition’.

16) Reference list: delete [see comments] in refs. 12 and 15. In ref. 17, the authors and title of ref. 19 are repeated.

17) Table 3, footnote: ‘independance’ should read ‘independence’.

18) Table 4, the allele frequencies column has a mistake for V153I (0.12-10:04 ?).

Discretionary Revisions (which the author can choose to ignore)

1) Page 6, first paragraph. The detection techniques reported in Posukh et al. might be described also here, since they have not been ‘previously described’ and the paper by Posukh et al. is currently only submitted.

2) When referring to the GJB2/GJB6 mutations as a whole, I would suggest to use the term ‘DFNB1 mutations’ instead (throughout the text).

3) Table 2. I would suggest to present the genotypes with two sequence variants in a first group, and those with only one sequence variant in a second group, with an empty row in between.
**What next?:** Accept after minor essential revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

NONE