Reviewer's report

Title: Variable expressivity of FGF3 mutations associated with deafness and LAMM syndrome

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Reviewer: Frei Klemens

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1. Major Compulsory Revisions:
Highly analogous results concerning the p.R95W mutation have been published previously. The expression pattern of p.R95W is reported in the paper to have 50% of cases (V1,V4, V8 and V10) as bi-CLA, in V7 unilateral CLA, in V4 and V5 not available and only in one case is a dysplastic SCC right side and 1and1/2 turn (Mondoni) left side (V3). V7 is reported as CLA left and reminescent basal turn right. In summary more than half of the inner ear structures are defined as a complete labyrinthine aplasia CLA. Only three inner ears are malformed. Although this frequency is moderately higher than the Somali family reported previously, the results are essentially identical.

The dental results reported in the paper should be confirmed by dental surgeons. Statements such as ‘absent syndromic features’ should be more carefully drafted as ‘minimal features’, if appropriate. The dental images shown for homozygous patients would support a description of ‘microdontia with conical teeth’. These data should be reevaluated.

The authors conclude ‘semi-dominance’ since 3 heterozygote carriers of p.R95W also have comparable, bilateral hearing loss. There are, however, a variety of other reasons why bilateral hearing loss can occur in such a family and these should be investigated.

In the absence of ligand-receptor crystal structures, the molecular modeling methodology employed can be helpful in predicting structural interactions but can deliver no definitive information on the effects of mutations on the binding of
ligands to glycosylated receptors. The conclusions should be modified appropriately.

The authors describe different mutations in FGF3 and postulate that some patients with FGF-related HL are potential candidates for CI-Implantation. They do not say which mutation. They do not state which mutation should be screened. This is important.

The data presented from the described families should be thoroughly checked between the table and associated diagrams. As written, the manuscript is difficult to follow as the data sets have become confused. In addition, some of the images presented also appear to have been duplicated.

2. Minor Essential Revisions:

P3, background li 2/3: It would be better to order the symptoms for LAMM in the order CLA, microtia and microdontia.

P3, background li3/4: In the background section the authors state that ‘In contrast, Fgf3 knockout mice have normal or mildly abnormal inner ears’ whereas in the discussion (p15), they state ‘….highly variable inner ear phenotype of Fgf3 knockout mice on a uniform genetic background’. The former erroneous and confusing statement should be removed from the abstract.

P3 methods li 3: the origin of the large families should be stated.

P3 methods li 4: How were the inner ear abnormalities detected? Anatomy, bony structures, nerves?

P3 results li 4/5: CLA in more than of the half inner ears is not ‘less severe’ but different.

P4: conclusions: One long sentence is too confusing: Construct two sentences, and rephrase ‘absent syndromic features’.

P5; li 2: references 1,2 Morton: Morton CC decribed a frequency for congenital deafness of 1.33 per 1,000 newborn in the United Kingdom and ‘1.86 per 1000 seems to be a reasonable overall estimate for the incidence at birth’. Smith stated: ‘Thus, at least one child in 1,000 is born with bilateral SNHL of at least 40 dB’. If the authors have no specific data for the Pakistani population, they should amend the quoted figures.

P5; li 9/10: ‘The etiology of CLA is usually unknown except for thalidomide exposure’. Tekin has described a reason for CLA-mutations in fgf3-why is this not mentioned?

P5; li 19: LAMM: The order should be CLA, microtia and microdontia.
P5; li 22: ‘revealed no cochlear or internal’. Complete labyrinthine aplasia is better.
P6; li 1: less severe is a relative, because labyrinthine aplasia is not less severe
P6; li 15: how is labyrinthine aplasia a mild phenotype?
P7; li 1: What is ‘the IRB’?
P7: mutation screening of fgf3+10: Only the primer design is mentioned, whereas
other methodology, PCR conditions, double strand sequencing etc are not
shown. If already published, references should be provided.
P8; li 8/9: Which examinations were performed? Otolarygolgical status,
micro-otoscopy, PTA, acoustic impedance (tymanometry), acoustic reflex
/stapedius reflex, ABN, OAE would be objective methods?
P8: CT and MRI also reveal middle ear structures and nerves, not only inner ear
structures
P8: is there any word recognition, function of the nerve, hearing aids as a criteria
for CI?
P9; li 1/2: For a better understanding of the possible role of modifier genes, it
should be mentioned that PKDF537 and 702 have no mutation in fgf3, if this is
the case.
P9; li 6: 162 ethnically matched... Are the Pakistani population intended here or
which origin do they have?
P9; li 16: conductive HL: There are many reasons for conductive HL. Have these
been excluded? If so, the authors should write ‘with normal otolaryngological
status, normal ear channels, normal tympanic membrane’ and exclude glue ears.
Otherwise the statement that a heterozygous mutation causes mild to moderate
degree of bilateral conductive HL cannot be taken seriously.
P9; li 18/19: three heterozygotes are mentioned: IV5, V6 and V9. 1. V6 het or
homo: In table 1 (carring homozygous FGF mutations) V6 is shown with no
ear-malformation, not available for microdontia and CT or MRI. 2). V-2 (13yr) is
shown as heterozygous in figure 2. These mistakes make cumbersome reading.
P10; li 22: is there a PTA? Does V2 having a conductive HL as the other
heterozygous carrier?
These data are very confusing! It is better to state clearly in the figure which
patients are homo or hetero.
P10; li 24: in four of six members:
table 1: V:1 Bi-CLA
V:4 Bi-CLA
V:7 Lt-CLA
V:8 Bi-CLA
V:10 Bi-CLA

So there are at least 5 affected: 4 Bi-CLA and 1 Uni-CLA?
P10/11: This description is very confusing. Figure b-c is family PKF295, 3e-g are family 817 and 887 mixed. 3d has a partial developed inner ear. Why didn’t the authors continue in alphabetical order? Interrupted by 3d?
To this reviewer, images 3d and 3n (right middle) appear to be the same image with a different contrast setting. Is there a reason why the authors show 3d and 3n? They should write that 3d and 3n is V-3 (V:3), but I’m guessing.
P11; li 3+15: MRI: 3i-j and 3l-m are which patients in which family?
P11; li 3/4: not only pneumatizations should be described but also ossicles of the middle ear, exclusion of cholesteatoma and other diseases.
P11; li 13: V7 is mentioned in text, is there also an image?
P13; li 7: why do the authors think recessive FGF3 mutations can involve ‘nearly non-syndromic deafness’? Equivocal teeth are described in V-8 and V-10, but is this really equivocal. Are there any measurements of dental and parameters for the Pakistani population, for reference? In V-10 it would be nice to see this better. It might be that these ears are conspicuous for Weerda I. Also, frontal images would be informative to see the position of the ears in correlation to the head.
P14; li 4: conductive HL: Have other reasons for conductive HL definitely been excluded to support the argument that a heterozygous mutation can lead to this?
P14; li 11: the statement ‘show isolated mild conductive hearing loss’ is supported by the references 36 ‘Audiometric examination revealed bilateral severe mixed type hearing loss (pure tone average on left ear was 95 dB and right ear was 85 dB)’ and reference 37: ADULT-syndrome: an autosomal-dominant disorder with pigment anomalies, ectrodactyly, nail dysplasia, and hypodontia. The paper abstract even states ‘We describe a family with at least seven living persons who are affected by an hitherto undescribed autosomal-dominant syndrome with variable expression, bearing close resemblance to the EEC syndrome and related disorders. The main manifestations are hypodontia and/or early loss of permanent teeth, ectrodactyly, obstruction of lacrimal ducts, onychodysplasia, and excessive freckling. We
propose the acronym ADULT (acro-dermato-ungual-lacrimal-tooth)-syndrome for this condition.

P14; li 16: reference 38 refers to high frequency HL ‘All affected patients had the unique phenotype of grossly enlarged molar teeth (globodontia) segregating with a high-frequency sensorineural hearing loss.’

In table 1, why is PKDF295 c.310c>t p.r104x exclusively associated with reference 9 (Tekin et al). Was this not also examined in this study? What next?: