Novel form of X-linked nonsyndromic hearing loss caused by a mutation in a type IV collagen gene

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INTRODUCTION

Hereditary hearing loss is one of the most common human diseases worldwide with an incidence of approximately 1 in 1000 newborns. Nonsyndromic hearing loss is extremely heterogeneous with more than 50 associated genes known to date. In most cases, non-syndromic hearing loss is inherited in an autosomal recessive pattern while X-linked forms are very rare accounting for only 1-5%.

RESULTS & DISCUSSION

Here, we present a three-generation family in which only males suffer from profound congenital hearing loss due to cochlear malformations while some females show a mild hearing impairment in later life (Fig. 1 and Fig. 2). The pedigree strongly suggests X-linked inheritance.

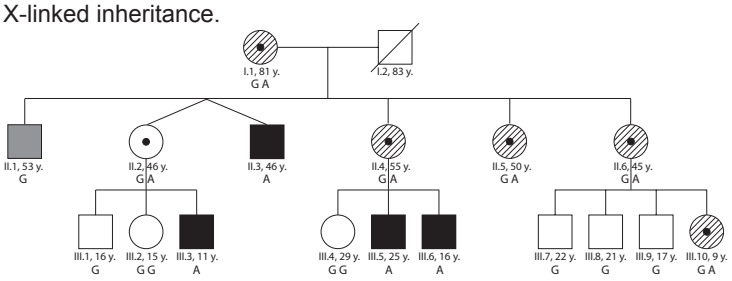

Figure 1: Pedigree of the family. Black squares indicate affected males with severe hereditary hearing loss, the gray square (II.1) indicates a male with acquired deafness, circles with black dots show mutation carriers who are healthy to date (unshaded) or who are affected mildly to moderately (shaded).

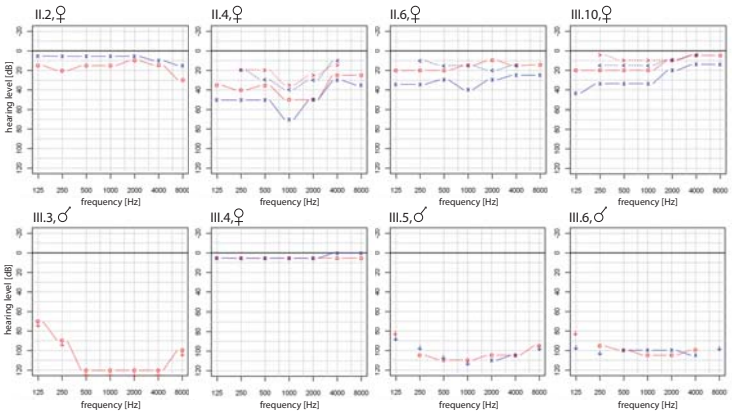

Figure 2: Representative pure tone audiometry of eight family members. The females II.2 and III.4 show regular thresholds at their current age. The females II.6 and III.10 show normal thresholds of their right ears and suffer from low grade conductive hearing loss on their left side. Female II.4 expresses bilateral moderate mixed hearing loss and an additional conductive component. Thresholds of patients III.3, III.5 and III.6 at their latest follow up showing only residual hearing in single frequencies (red – right side, blue – left side, circles and crosses – air conduction, arrow heads – bone conduction, vertical arrows – frequency not heard).

After exclusion of the known X-linked deafness genes *POU3F4* and *PRPS1*, next generation sequencing of the whole X-chromosomal exome was performed in the index patient, his mother and his affected cousin. Filtering and comparison of about 10.000 exonic variants per person revealed only a few shared unknown variants in which a missense mutation in a type IV collagen gene perfectly co-segregated with the disease in the family (Fig. 1).

Bioinformatic analyses including prediction of functional effects, splice site prediction as well as in silico analysis of protein stability and structure confirmed the causality of the detected variant (Fig. 3).

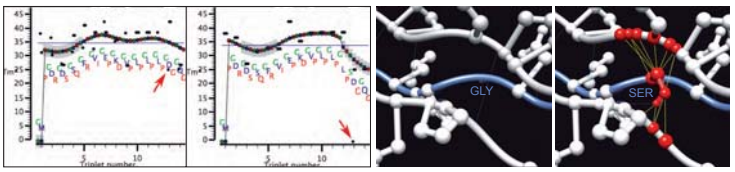

Figure 3: Structural comparison of wild-type collagen with the detected variant. Left subfigures illustrate estimated melting temperatures in degrees Celsius along the type IV collagen amino acid sequence. The respective mutation site is highlighted with an arrow. The mutation seriously affects the melting temperature of the natural model. Three-dimensional models (right subfigures) of the heterotrimeric collagen chains (one in blue, two in white): Atoms and bonds are colored in red in case they clash or have unfavorable contacts with atoms of the other helices (van der Waals overlap greater than 0.6 angstroms). The pairings of clashing atoms are indicated by yellow lines.

In situ hybridization in zebrafish embryos (Fig. 4) and immuno-staining in the mouse inner ear (Fig. 5) demonstrated expression of the type IV collagen in the otic vesicle of zebrafish and in the spiral ligament of the murine ear, respectively. Expression data in combination with the cochlear malformations visible in the male patients provide evidence for an essential role of the type IV collagen in normal ear development and function. Although mutations in type IV collagen genes are known to be associated with Alport syndrome, a nephropathy often combined with hearing loss, there is no evidence for the presence of an Alport syndrome in the examined family.

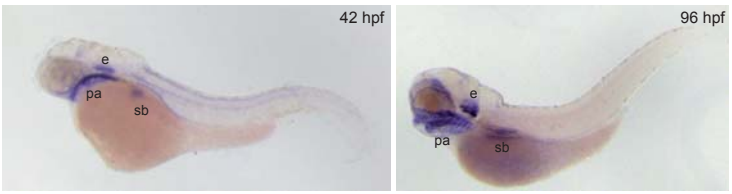

Figure 4: Type IV collagen expression in the zebrafish embryo. Whole-mount in situ hybridization on zebrafish embryos was performed in different developmental stages (e: ear, hpf: hours post fertilization, ot: otic vesicle, pa: pharyngeal arch, sb: swim bladder).

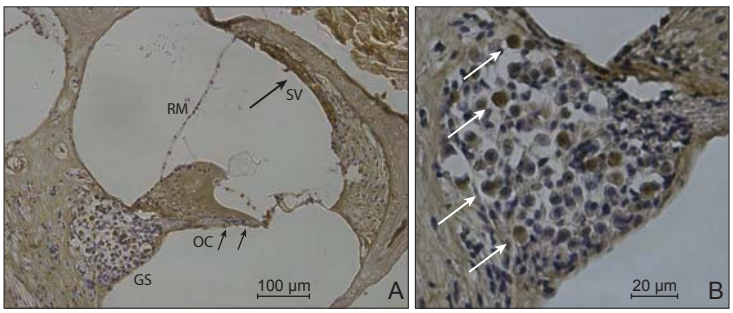

Figure 5: Type IV collagen expression in the mouse inner ear. A) Thin sections of a de-mineralised murine inner ear were incubated with rabbit polyclonal Col4A6 antibody M137. Type IV collagen is strongly expressed at membranous and osseous structures at the stria vascularis of the spiral ligament (black arrow) which were more intensely stained as compared to the basilar membrane underlying the organ of Corti (small black arrows). GS – ganglion spirale, OC – organ of Corti, RM – Reissner's membrane, SV – stria vascularis. B) At higher magnification, a very distinct and pronounced reactivity was seen in a subgroup of ganglia cells of the ganglion spirale (white arrows).

CONCLUSION

In conclusion, our results suggest one of the X-chromosomal collagen genes as being the fourth gene associated with X-linked non- syndromic hearing loss.

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