ONLINE MUTATION REPORT

Autosomal recessive postlingual hearing loss (DFNB8): compound heterozygosity for two novel *TMPRSS3* mutations in German siblings

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J Med Genet 2007;**44**:e81 (http://www.jmedgenet.com/cgi/content/full/44/6/e81). doi: 10.1136/jmg.2007.049122

Mutations in the transmembrane protease, serine 3 (*TMPRSS3*) gene, encoding a transmembrane serine protease, cause autosomal recessive deafness childhood (DFNB8) or congenital onset (DFNB10). *TMPRSS3* mutations have been mainly identified in patients from Asian and Mediterranean countries and seem to be a rare finding in the Northern European population so far. The identification of two novel pathogenic *TMPRSS3* mutations (c.646C→T − R216C; c.916G→A − A306T) is described in four affected siblings of German origin with postlingual hearing loss, treated by bilateral cochlear implantation with good results. Although *TMPRSS3* mutations are supposed to be a rare cause of autosomal recessive hearing loss, in families with postlingual disease onset *TMPRSS3* is the most favourable candidate gene after exclusion of *GJB2* mutations.

Autosomal recessive non-syndromic deafness is the most common type of inherited hearing impairment, with an account of approximately 80% cases. Over 50 autosomal recessive non-syndromic hearing loss loci have been mapped, but to date only about half of the genes have been isolated (hereditary hearing loss homepage: http://webh01.ua.ac.be/hhh/). Among these, connexin 26 (*GJB2*) is responsible for a large number of cases (30–60%).[1–3] The typical phenotype of autosomal recessive non-syndromic hearing loss is profound prelingual.

By contrast, postlingual forms seem to be either autosomal dominant or maternally inherited because of mitochondrial mutations. Autosomal recessive hearing loss with childhood onset is a rare clinical finding.[4] Exceptions are caused by mutations in the transmembrane protease, serine 3 (*TMPRSS3*) gene on chromosome 21q22,[5 6] which can lead to autosomal recessive prelingual (DFNB10) as well as to postlingual deafness (DFNB8).[7] *TMPRSS3* encodes a transmembrane serine protease, which is inter alia expressed in the organ of Corti, the stria vascularis and in the spiral ganglion, and activates the epithelial sodium channel.[8 9] Although the mutations of *TMPRSS3* have been identified in patients with hearing loss from Asian and Mediterranean countries, in the Caucasian population the proportion of patients with hearing loss caused by *TMPRSS3* mutations seem to be small, with an estimated frequency of <1%.[10 11]

In this study, we describe the detection of two new missense mutations (R216C and A306T) in *TMPRSS3* in a family of German origin.

PATIENTS AND METHODS

Patients

Our family included four affected siblings, two females and two males, with postulated autosomal recessive postlingual non-syndromic hearing loss as well as six non-affected brothers and sisters (fig 1A). The non-affected parents were unrelated, and of German origin. The first onset of hearing loss was reported at an age of 6 years, with progression to deafness by about 20 years in all affected individuals. In the affected siblings, *GJB2* mutations were first excluded. As controls, we screened 100 normal hearing probands of German origin.

DNA studies

Linkage analysis

Blood samples from 11 family members were obtained after informed consent was given. Genomic DNA was isolated from blood samples using standard protocols. For haplotype analysis, polymorphic microsatellite markers spanning the *TMPRSS3* locus were used (centromere–D21S266–*TMPRSS3*–D21S1411–D21S1890–telomere). The PCR products were run on an ABI 3130 automatic sequencer (Applied Biosystems, Darmstadt, Germany).

Mutation analysis

All coding exons and the exon–intron boundaries of the *TMPRSS3* gene were amplified by PCR using standard protocols (primer sequences were designed by using the Primer3 software: http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). PCR products were sequenced using the Big Dye Terminator Cycle Ready Reaction Kit V.1.0 (Applied Biosystems) on an ABI 3130 sequencer. The initial analysis included one affected person and the mother. When a sequence variant was identified, direct sequencing was performed in all family members to verify its segregation with the phenotype.

To allow rapid genotyping of controls, we developed PCR-based restriction fragment length polymorphism assays for the identified mutations R216C and A306T. In both assays, 5 µl of the PCR product was digested with 5 U of NsiI (R216C mutation) and 5 U of MslI (A306T mutation), according to the manufacturer's instructions (New England Biolabs, Frankfurt, Germany). Digestion products were electrophoresed on 3% agarose gels.

RESULTS AND DISCUSSION

In a large German family with four siblings affected by postlingual non-syndromic hearing loss, we searched for *TMPRSS3* mutations as this gene is known to cause autosomal recessive hearing loss of postlingual onset.

Firstly, the segregation of three highly polymorphic microsatellite markers flanking *TMPRSS3* was studied for linkage with the phenotype. We reconstructed the haplotypes of all available family members (fig 1A). One recombination was found (person II.5) between the two tested markers D21S266

Abbreviations: *TMPRSS3*, transmembrane protease, serine 3 gene

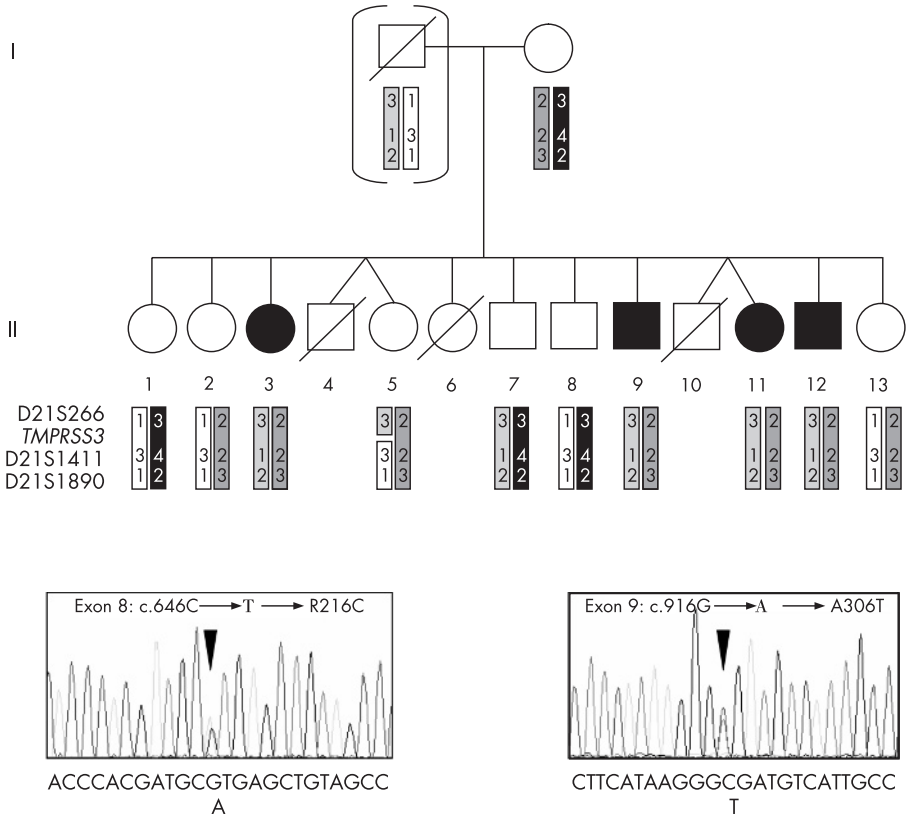

Figure 1 (A) Family pedigree and results of linkage analysis with polymorphic microsatellite markers spanning the transmembrane protease, serine 3 (*TMPRSS3*) locus on chromosome 21q22 (paternal DNA was not available, therefore the paternal haplotypes were delineated from the typing results in his children). (B) Direct sequencing of exons 8 and 9 of *TMPRSS3* in patients II.3 and II.9 showed compound heterozygosity of two new missense mutations (sequencing was performed using the reverse primers).

and D21S1411, but direct sequencing showed that the recombination must have occurred telomeric from the coding sequence of *TMPRSS3*. However, these microsatellite typing results were consistent with linkage to the *TMPRSS3* locus.

By direct sequencing of the exons and intron–exon boundaries of *TMPRSS3*, we identified compound heterozygosity of two new mutations cosegregating in the four affected family members (fig 1B).

The mutation in exon 8 (c.646C→T) results in an arginine to cysteine substitution at codon 216 (R216C) and affects the same codon as the known pathogenic mutation c.647G→T (R216L).[9] The second mutation was found in exon 9 (c.916G→A) and leads to an alanine to threonine substitution (A306T). Both missense mutations change evolutionary highly conserved amino acids. They were not found in 200 control chromosomes of German origin. Analysis of the mutant *TMPRSS3* harbouring a R216L mutation showed that it failed to undergo proteolytic cleavage and could not activate the epithelial sodium channel.[9]

Apart from the two new identified pathogenic mutations, we detected two known polymorphisms cosegregating with the maternal pathogenic mutation (intron 7: IVS7-3ins(TA), rs2839500; exon 8: c.757A→G, p.I253V, rs28437266[5]).

Owing to the large number of known genes associated with hearing loss, the identification of the underlying genetic cause of autosomal recessive non-syndromic hearing loss is difficult in cases where *GJB2* mutations have been excluded. Although *TMPRSS3* mutations account only for a small number of Caucasian patients with hereditary hearing loss,[10 11] we think that in the cases of postlingual disease onset and possible autosomal recessive inheritance, *TMPRSS3* is the most favourable candidate gene. The detection of compound heterozygosity for mutations in *TMPRSS3* in our family impressively illustrates that testing of rare disease genes is worthwhile also in cases without evidence for consanguinity.

DATA ACCESS

GenBank: AB038157.

ACKNOWLEDGEMENTS

We thank the family who participated in this study.

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Competing interests: None declared.

Informed consent has been obtained from patients for publication of their details in this paper.

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Received 11 January 2007
Revised 2 March 2007
Accepted 9 March 2007

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