OTOF mutations revealed by genetic analysis of hearing loss families including a potential temperature-sensitive auditory neuropathy allele

Running Title: A potential OTOF temperature-sensitive allele

Renee Varga1,2, Matthew R. Avenarius3, Philip M. Kelley1, Bronya J. Keats4, Charles I. Berlin5, Linda J. Hood5,7, Thierry G. Morlet5,8, Shanda M. Brashears5,9, Arnold Starr6, Edward S. Cohn1, Richard J. H. Smith3, and William J. Kimberling1

1) Center for Hereditary Communication Disorders, Boys Town National Research Hospital (BTNRH), Omaha, NE, USA; 2) Current affiliation: National Human Genome Research Institute, NIH, Bethesda, MD, USA; 3) Molecular Otolaryngology Research Laboratory, Department of Otolaryngology, University of Iowa Hospitals and Clinics, Iowa City, IA, USA; 4) Department of Genetics, LSU Health Sciences Center, New Orleans, LA, USA; 5) Department of Otorhinolaryngology, LSU Health Sciences Center, New Orleans, LA, USA; 6) Department of Neurology, University of California, Irvine, CA, USA; 7) Current affiliation: Hearing and Speech Sciences Department, Vanderbilt Bill Wilkerson Center, Vanderbilt University Medical Center, Nashville, TN, USA; 8) Current affiliation: Auditory Physiology and Psychoacoustics Laboratory, A.I. duPont Hospital for Children, Wilmington, DE, USA; 9) Current affiliation: Audiology Clinic, A.I. duPont Hospital for Children, Wilmington, DE, USA.

Corresponding Author: Philip M. Kelley, Ph.D., BTNRH Genetics Dept. 555 N. 30th St. Omaha, NE 68131. Phone: 402 498 6385 Fax: 402 498 6331 E-mail: Kelley@boystown.org

Key Words

OTOF
DFNB9
Otoferlin
Auditory Neuropathy
Hearing Loss
Temperature-sensitive

Word count = 3,831
Abstract

Introduction. The majority of hearing loss in children can be accounted for by genetic causes. Nonsyndromic hearing loss accounts for 80% of genetic hearing loss in children with mutations in DFNB1/GJB2 by far the most common cause. Among the second tier genetic causes of hearing loss in children are mutations in DFNB9/OTOF (OMIM: 603681).

Methods. Sixty-five recessive nonsyndromic hearing loss families were screened by genotyping for association with the DFNB9/OTOF gene. Families with genotypes consistent with linkage or uninformative for linkage to this gene region were further screened for mutations in the 48 known coding exons of otoferlin.

Results. Eight OTOF pathologic variants were discovered in six families. Of these, Q829X was found in two families. Twenty-three other coding variants believed to have no pathology were also noted. A previously published missense allele I515T was found in the heterozygous state in an individual who was observed to be temperature-sensitive for the auditory neuropathy phenotype.

Conclusions. Mutations in OTOF cause both profound hearing loss and a type of hearing loss where otoacoustic emissions are spared called auditory neuropathy.
Introduction

The majority of hearing loss in children can be accounted for by genetic causes. Nonsyndromic hearing loss accounts for 80% of genetic hearing loss in children with mutations in DFNB1/GJB2 by far the most common cause.[1] Among the second tier genetic causes of hearing loss in children are mutations in DFNB9/OTOF (OMIM: 603681). This type of hearing loss is further complicated in that at an early stage it may present itself as an auditory neuropathy and thus evade detection by newborn hearing screening based on otoacoustic emissions testing. [2-4]

Auditory Neuropathy/Auditory Dys-synchrony (AN/AD) is a unique type of hearing loss diagnosed when tympanograms are normal, and acoustic reflexes (AR) and auditory brainstem response (ABR) are absent or severely abnormal but, outer hair cell (OHC) function is normal as indicated by the presence of otoacoustic emissions (OAE) and/or cochlear microphonics (CM). These test results indicate that the auditory pathway up to and including the OHC is functioning but the auditory signal is not transmitted to the brainstem, suggesting that the lesion lies at the level of the inner hair cells (IHC), the IHC synapse to the afferent nerve fibers, or the auditory nerve itself. Individuals with AN/AD can have various degrees of hearing loss as measured by pure-tone audiometry. However, they generally have disproportionately poor speech understanding. In contrast to persons with non-AN/AD hearing loss, hearing aids may provide little help in speech understanding in most individuals with AN/AD. Cochlear implantation has been shown to help with speech understanding in some cases of AN/AD [5-10] but others have not had favorable results. [11, 12]

The term “auditory neuropathy” was first coined in 1996.[13] However, many early reports describe cases now thought to have been AN/AD. [14-16] At the time, such cases were considered paradoxical because test findings were inconclusive or contradictory. However, advances in the measurement of OHC function allowed further characterization of this condition. Approximately 50% of AN/AD patients have no defined etiology. [17] These patients are generally non-syndromic and some pedigrees suggest a recessive pattern of inheritance based on the presence of at least two affected siblings and unaffected parents. Recently mutations in OTOF, the gene encoding otoferlin were found to cause a non-syndromic recessive AN/AD.[2-4] There have been several reports of mutations in OTOF associated with non-syndromic recessive hearing loss (NSRHL). [18-23] None of the NSRHL reports defined the status of the OHC in affected individuals, so it could not be determined whether these individuals had AN/AD.

In adult mouse cochlea, otoferlin mRNA is detectable in the IHCs but is not evident in OHCs. [23] Several isoforms of otoferlin exist due to variable start sites and alternative splicing. At least two other members of this gene family have been found in mammals: DYSF encoding dysferlin and MYOF encoding myoferlin. [24] Dysferlin was recently found to be involved in membrane repair. [25] Otoferlin, dysferlin, and myoferlin are predicted to be signal-anchor membrane proteins with the greater part of the protein including the N-terminus of the protein facing the cytoplasm anchored by the C-terminal transmembrane domain. [26, 27] They contain multiple C2 domains, and all bear homology to the synaptotagmins, proteins involved in synaptic vesicle fusion. [28]

We report here the genetic screen of 65 hearing loss families, the discovery of novel otoferlin mutations and corresponding clinical data on hearing loss in families with pathological
mutations. One of these mutant alleles is of particular interest because the phenotype associated with this allele is temperature sensitive.

Materials and Methods

**Patient sample and medical information**

DNA samples and medical information were collected under IRB protocols that have been approved by the respective institutions. Sampling included the proband, and if possible, all full biological siblings, both biological parents, and all biological grandparents. In some cases, research participants were asked to undergo complete physical exams and other appropriate testing, including audiologic, vestibular, neurologic, and ophthalmologic testing. Audiologic testing included air- and bone-conduction pure-tone audiometry, ABR, tympanometry, AR thresholds, OAE, OAE suppression, speech awareness threshold (SAT) and/or speech reception threshold (SRT) testing. The following scale was used to classify the degree of hearing loss using pure-tone audiometry: 0-20 dB HL as normal, 21-40 dB HL as mild, 41-60 dB HL as moderate, 61-80 dB HL as severe, and above 80 dB HL as profound.

**Genotyping and mutation screening**

All families in this study were previously screened for DFNB1/GJB2 mutations. In the Nebraska group of 47 NSRHL families (recruited at BTNRH or LSU), genotyping of the chromosomal region around the OTOF locus was done as previously described.[2] To determine if a difference exists between the patient's DNA and the control OTOF BAC DNA (RP11-638P8, Research Genetics, Inc.), mutation detection enhancement (MDE) heteroduplex analysis was performed as previously described with slight modifications. [29] PCR products were amplified from both genomic sample DNA and OTOF BAC control DNA, mixed, heated at 95°C for 3 minutes and then cooled to 25°C over a 45 minute period. The re-annealed reaction products were then electrophoresed at 850V for 18-24 hours, depending on the size of the fragment. Following electrophoresis, the bands were visually assessed using ethidium bromide staining. PCR primers used to amplify OTOF exons and flanking sequence have been previously described. [2]

Denaturing High-Performance Liquid Chromatography (DHPLC) using the Transgenomic WAVE DNA Fragment Analysis System was also used to identify mutations according to the manufacturer’s directions (Transgenomic, Inc.).

In the Iowa group (families recruited at the University of Iowa), 18 DFNB1 negative NSRHL families were analyzed for allele status with STRP markers (D2S405, D2S158, and D2S1360). Three families with markers segregating consistent with linkage or uninformative for linkage were further screened using SSCP for all 48 known OTOF exons. Mutations recognized by SSCP were then identified by sequencing. Families where only one mutation was detected were screened by sequencing all 48 known OTOF exons.

DNA samples showing a positive heteroduplex pattern by either the MDE gel-based method, the DHPLC method or SSCP were sequenced using either the ABI Prism™ BigDye Terminator Cycle Sequencing Ready Reaction kit or Beckman Coulter™ CEQ Dye Terminator Cycle Sequencing Quick Start kit. Analysis of the sequence data was performed using Sequence Analysis 3.4, the Lasergene suite (DNASTAR, Inc) and the Wisconsin Package (Pharmacopeia, Inc). Mutations were checked in unrelated normal-hearing control individuals using restriction enzyme digestion or heteroduplex gel/WAVE DHPLC and DNA sequencing if no restriction
enzyme was available. The control population consisted of Americans of European Caucasian
descent with no report of hearing problems. In this study all families with individuals affected with
hearing loss were also American of European Caucasian descent except one family from Great
Britain. Mutations corresponding to the coding region are numbered as in the human brain
otoferlin long form AF183185.1 beginning with the starting methionine AUG, A as number 1.
Splice-site mutations are designated by their adjacent exon. [23]

Results

Mutations were revealed during two independent screens of NSRHL families. In the
Nebraska group, STRP genotyping of the OTOF locus was performed on 47 families (38
NSRHL families and 9 Non-Syndromic Recessive Auditory Neuropathy (NSRAN) families), 17
(14 NSRHL and 3 NSRAN) of whom were consistent with linkage to the OTOF locus by
haplotyping and were therefore included in the genetic screening. Sixteen families were
uninformative for linkage (12 NSRHL and 4 NSRAN). Fourteen families (12 NSRHL and 2
NSRAN) were informative and not consistent with linkage. The 17 families consistent for
linkage and the 16 uninformative for linkage were included in the mutation screen. Seven
mutations believed to be pathogenic were found in five families in this group (Table 1 and Figure
1).
Table 1  OTOF mutations including mutations from genetic screening of 65 NSRAN and NSRHL families (in bold).

<table>
<thead>
<tr>
<th>Exon or IVS</th>
<th>Mutation</th>
<th>Codon</th>
<th>Controls # of chromosome</th>
<th>Family</th>
<th>Diagnosis</th>
<th>origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>709 C&gt;T</td>
<td>R237X</td>
<td>0/36</td>
<td>NSRHL</td>
<td>UAE</td>
<td></td>
<td>[19]</td>
</tr>
<tr>
<td>8 IVS</td>
<td>IVS 8-2 A&gt;G</td>
<td></td>
<td>0/218</td>
<td>NSRHL</td>
<td>India</td>
<td></td>
<td>[23]</td>
</tr>
<tr>
<td>15</td>
<td>1469 C&gt;A</td>
<td>P490Q</td>
<td>0/220</td>
<td>NSRHL</td>
<td>Turkey</td>
<td></td>
<td>[21]</td>
</tr>
<tr>
<td>15</td>
<td>1544 T&gt;C</td>
<td>I515T</td>
<td>0/178, 0/220</td>
<td>TS-NSRAN, NSRHL</td>
<td>USA, Turkey</td>
<td></td>
<td>[21]</td>
</tr>
<tr>
<td>16</td>
<td>1651 delG</td>
<td></td>
<td></td>
<td>NSRAN</td>
<td>USA</td>
<td></td>
<td>[2]</td>
</tr>
<tr>
<td>17</td>
<td>1886_1887 insA</td>
<td>K629fs</td>
<td>0/188</td>
<td>NSRAN</td>
<td>USA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 IVS</td>
<td>IVS 18+1 G&gt;T</td>
<td></td>
<td>0/188</td>
<td>NSRAN</td>
<td>USA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>2122 C&gt;T</td>
<td>R708X</td>
<td>0/100</td>
<td>NSRHL</td>
<td>Spain</td>
<td></td>
<td>[3]</td>
</tr>
<tr>
<td>21</td>
<td>2348 delG</td>
<td>G783fs</td>
<td>0/184</td>
<td>NSRAN</td>
<td>USA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>2381 G&gt;A</td>
<td>R794H</td>
<td>0/160</td>
<td>NSRHL</td>
<td>USA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>2485 C&gt;T</td>
<td>Q829X</td>
<td>0/172, 0/400</td>
<td>NSRAN, UK, USA, Spain, Cuba</td>
<td>NSRHL Druze/Israel</td>
<td>[3] [20]</td>
<td></td>
</tr>
<tr>
<td>24 IVS</td>
<td>IVS 24+1 G&gt;A</td>
<td></td>
<td>0/240</td>
<td>NSRAN</td>
<td>USA</td>
<td></td>
<td>[18]</td>
</tr>
<tr>
<td>26</td>
<td>3032 T&gt;C</td>
<td>L1011P</td>
<td>0/100</td>
<td>NSRAN</td>
<td>Turkey</td>
<td></td>
<td>[4]</td>
</tr>
<tr>
<td>28 IVS</td>
<td>IVS 28-2 A&gt;C</td>
<td></td>
<td>0/184</td>
<td>NSRAN</td>
<td>UK</td>
<td></td>
<td>[3]</td>
</tr>
<tr>
<td>36</td>
<td>4275 G&gt;A</td>
<td>W1425</td>
<td>X</td>
<td>NSRAN</td>
<td>Spain</td>
<td></td>
<td>[3]</td>
</tr>
<tr>
<td>36 IVS</td>
<td>IVS 36+2 T&gt;G</td>
<td></td>
<td>0/100</td>
<td>NSRAN</td>
<td>Spain</td>
<td></td>
<td>[3]</td>
</tr>
<tr>
<td>37</td>
<td>4491 T&gt;A</td>
<td>Y1497</td>
<td>X</td>
<td>NSRHL</td>
<td>Lebanon</td>
<td></td>
<td>[22]</td>
</tr>
<tr>
<td>39 IVS</td>
<td>IVS 39+1 G&gt;C</td>
<td></td>
<td>0/188</td>
<td>NSRAN</td>
<td>USA</td>
<td></td>
<td>[2]</td>
</tr>
<tr>
<td>44</td>
<td>5473 C&gt;G</td>
<td>P1825A</td>
<td>0/200</td>
<td>NSRHL</td>
<td>Spain</td>
<td></td>
<td>[20]</td>
</tr>
<tr>
<td>48</td>
<td>5860_5862 delATC</td>
<td>I1995de</td>
<td>0/100</td>
<td>NSRAN</td>
<td>Spain</td>
<td></td>
<td>[3]</td>
</tr>
<tr>
<td>48</td>
<td>6014 G&gt;A</td>
<td>R1939Q</td>
<td>0/188</td>
<td>NSRAN</td>
<td>USA</td>
<td></td>
<td>[2]</td>
</tr>
<tr>
<td>48</td>
<td>6158 C&gt;G</td>
<td>P1987R</td>
<td>0/188</td>
<td>NSRAN</td>
<td>USA</td>
<td></td>
<td>[2]</td>
</tr>
</tbody>
</table>

In the Iowa group, 18 NSRHL families were screened with markers linked to OTOF. None of the Iowa families had been previously diagnosed with auditory neuropathy. Of these 18 families all were informative for linkage but only 3 families were consistent for linkage to OTOF. These families were screened by SSCP. A single missense mutation deemed pathogenic was identified in family 53510 in this group (see Table 1 and Figure 1). An audiologic summary is given for each family in Table 2.
We found a high degree of neutral variation within the subject population during the mutation screen. Twenty-three coding polymorphic changes were noted (alleles in bold found in the Spanish population). [20] Eleven of these were missense alleles (158C>T, A53V; 244C>T, R82C; 1723G>A, V575M; 2317C>T, R773S; 2317C>T, R773S; 2464C>T, R822W; 2464C>T, R822W; 3247G>C, A1083P; 3247G>C, A1083P; 3966C>G, D1322E; 4874G>A, V1625M; 4874G>A, V1625M; 4936C>T, P1646S; 5663G>A, G1888D). Twelve of these were silent alleles (372A>G, T124T; 945G>A, K315K; 1926C>T, N642N; 2022C>T, D674D; 2025G>A, E675E; 2580C>G, V860V; 2736G>C, L919L; 3189G>A, A1063A; 4677G>A, V1559V; 4767C>T, R1589R; 5391C>T, F1797F; 5655C>T, R1885R).

Probably the most unusual family of the group is 3467, a temperature-sensitive AN/AD family with two affected siblings. The variant 1544T>C, I515T was found heterozygously in the father and the two affected children of this temperature-sensitive NSRAN family. The maternal mutation is still unknown.

When afebrile, individual 3467-1 audiogram showed a mild low-frequency hearing loss and speech comprehension was below the 10th percentile in both quiet and noise. Tympanometry was normal and AR were absent. ABR was abnormal, but CM were present. On two occasions, testing was performed during febrile illness. Her core temperature was defined approximately 2 hours before testing. At a temperature of 38.1°C, her pure-tone thresholds decreased to profound deafness in the low frequencies, rising to severe hearing loss in the high frequencies. SAT was at 80 dB nHL, but she was unable to repeat any of the test spondee words. Tympanometry and
OAE were normal, but AR and ABR were absent. With a temperature of 37.8°C, she was tested again and showed a mild-to-moderate hearing loss and 0% speech comprehension. ABR and OAE were not tested. The following day her auditory functions returned to normal after her fever abated. She has reported to her parents that her hearing becomes affected “suddenly” when she is febrile.

Individual 3467-2 was examined twice when afebrile. He has a mild low-frequency hearing loss, normal tympanograms, absent AR and abnormal ABR. CM and OAE were present bilaterally. We were unable to test 3467-2 when febrile, but his parents report that under those conditions he experienced hearing loss similar to his sister. OAE suppression was tested to determine medial olivocochlear (MOC) neuron integrity. Activation of these neurons by presenting sound to the contralateral cochlea will induce a suppression of ipsilateral OAEs.[30] Patients with AN/AD cannot suppress their OAE during this test. [31] As expected, his OAE were not suppressed, which indicates that the sound signal reached the OHC, but not the efferent MOC neurons that feed back to suppress the OHC. We have tested the parent carrying the I515T mutation and no abnormality of pure tone threshold, speech comprehension in quiet and noise, ABR, AR, and OAE were identified. The clinical details of this family have been published. [32]

Both OTOF pathologic alleles have been found in family 3456. The maternally inherited mutation is a novel splice-site mutation, IVS28 –2 A>C. The mutation inherited through their father is a previously published nonsense mutation 2485 C>T, Q829X, found in a group of Spanish families and one Cuban family. [3, 20] Family 3456 is a Caucasian family from England with no known Hispanic ancestry with two sons both having AN/AD. Pregnancy and birth history were unremarkable for the two affected boys except for slight jaundice in the oldest, individual 3467-1. Motor milestones were met at an appropriate age. As is consistent with AN/AD, ABR was absent and OAE were present. Both boys have profound hearing loss and corner audiograms. OAE suppression was tested in individual 3467-1 and, as expected, his OAEs were not suppressed, but were instead increased in amplitude. Vestibular function testing in the oldest indicated that there may be a slight hypofunction on the left side. The youngest was not tested for vestibular function. CTs were normal in both boys as well as an MRI in the oldest. Both boys have had positive experiences with their cochlear implants and consider them to be beneficial.

Both mutations in family 3466 result in a frameshift. The maternally inherited mutation in exon 17 contains an insertion 1886_1887insA (K629fs). The paternally inherited mutation is a deletion in exon 21, 2348delG (G783fs). This family has two affected children, a boy (3466-1) and a girl (3466-2) with normal OAE and absent ABR. Birth histories were uneventful and developmental milestones, except speech, were attained at a normal age. At age 5 years (3466-1) and age 7 years (3466-2), the children underwent neurological exams, which were normal. However 3466-1 may also have a vestibular neuropathy or hypoactive vestibular function since no nystagmus developed after spinning in a chair and his father reported that the child spins and never seems to fall down.

Family 3539 consists of two affected children with normal tympanometry, OAE and CM, but absent AR and ABR. The daughter was born prematurely at 32 weeks gestation; otherwise the birth histories were unremarkable. A splice-site mutation, IVS18+1 G>T, was found in the maternal allele.
The Hispanic nonsense mutation 2485 C>T, Q829X has been found heterozygously in family 3450. This family has Mexican ancestry. Both affected children are “typical” of NSRAN, in that OAE and CM are present, but ABR and AR are absent.

In the Iowa group of families, an OTOF mutation was found in one family. There were three affected children in family 53510, all heterozygous for 2381G>A, R794H. Two of these individuals had a profound hearing loss while one had a severe-to-profound hearing loss. All three individuals had a corner audiogram.

Discussion

Seven OTOF mutations were discovered and believed to be pathogenic in five NSRAN families in the Nebraska group. One missense mutation was found in the Iowa group of NSRHL families. It is possible that the previously published OTOF NSRHL families were actually NSRAN families, however proving this could be difficult due to the fact that, in many cases, OHC function has been observed to decline with age. The mutation found in the Iowa group of families reported in this study may also have been NSRAN for the same reason. Recent studies, including this work, indicate that all individuals with OTOF pathological variants in both the maternal and paternal alleles will present with auditory neuropathy as young children if comprehensively tested (including OHC function). [2-4] In this report, two families were heterozygous for the nonsense allele 2485C>T, Q829X, found recurrently in Spanish families. [3, 20] Two frameshift, two splice-site, and two missense mutations have also been found (table 1). One of the missense alleles, 1544T>C, I515T is associated with a temperature-sensitive NSRAN phenotype. It was previously described in cis with another missense mutation 1469C>A, P490Q not detected in the families reported here. [21]

The nonsense mutation 2485C>T, Q829X appears to be the most common otoferlin mutant allele discovered to date ([3, 20] and this report). In the Spanish population it is the third most common cause of genetic hearing loss in children. [3] Two splice-site mutations, IVS28 -2 A>C and IVS 18 +1 G>T, were discovered but their consequences have not been investigated. A single base insertion 1886_1887 insA, K629fs and single base deletion 2348 del G, G783fs have been detected. Both of these mutations are predicted to cause a frameshift with premature termination, which should result in mRNA destabilization via nonsense-mediated mRNA decay. [33]

The two missense mutations occur at amino acids that are conserved by identity in human, mouse, chicken and zebrafish otoferlin. Missense mutations were considered pathological if, 1) their segregation was consistent with affected status, 2) if no such variant was found in the control population and 3) the reference amino acid was conserved across vertebrate lines. The I515T is discussed in detail below. The arginine to histidine change, R794H, seen in family 53510 occurs as part of a coiled coil region identified by the SMART/COILS algorithm [34] and [35] that extends from otoferlin residues 792-820. This is a conservative change as both arginine and histidine are polar basic amino acids. However, arginine is more hydrophilic than histidine and differs significantly structurally. Coiled coil domains are characterized by alpha-helices that interact with one another potentially forming a “Peptide Velcro” interaction with other proteins.[36] Weakening the hydrophilic interaction in such a structure could affect an important protein-protein interaction disrupting otoferlin function. Marker analysis for the 53510 family was consistent and informative for linkage at DFNB9. None of the mutations considered pathologic was detected in a hearing control population of at least 80 individuals.
This is the second study to discover a high degree of polymorphic variation at the OTOF locus. All the polymorphisms found in the Spanish population were also found in the population described here and fifteen additional neutral variants were discovered. The primary criterion for describing a variant as neutral was the detection of that mutation in a set of normal hearing controls.

The proband in family 3467 has abnormal ABR, present OAE and relatively normal hearing until she becomes febrile, when OAE remain normal but hearing degrades and ABR worsens from abnormal with unidentified waves I-III and delayed latency of the wave IV-V complex to being totally absent. The amount of decline in hearing in the daughter is dependent on the degree of fever. A mild-to-moderate hearing loss was present during a fever of 37.8°C and a profound hearing loss was present during the 38.1°C fever.

Only one mutation has been found in family 3467 thus far, 1544T>C, I515T. This amino acid is conserved in human, mouse, chicken and zebrafish otoferlin. It is a missense mutation occurring in the otoferlin C2C domain that was previously published in a consanguineous family from Eastern Turkey with profound prelingual hearing loss. No mention was made of a temperature associated phenotype in this family. The affected members of the family from Eastern Turkey are homozygous for the I515T missense mutation and another missense mutation, 1469C>A, P490Q inherited in cis.

Both the P490Q and I515T mutations in the Turkish kindred are found in the C2C domain (third C2 domain), which is predicted to bind calcium. The alignment of otoferlin and otoferlin-related proteins revealed remarkable conservation of amino acids within the human and mouse C2C domains. Mirghomizadeh and co-workers predicted either of these two mutations would severely disrupt the structure of the C2C domain with the I515T mutation resulting in the creation of a new myristylation site. Temperature-sensitive mutations have been previously recognized in several human genetic diseases.

Eight OTOF mutations were found in 6 families in this study. In previous studies 4 mutations were found in three families and 10 mutations were found in six families. All other OTOF mutations described are in consanguineous families or in Spanish families homozygous for Q829X. OTOF is a large gene with multiple isoforms coded by at least 48 known exons. The screening of this gene focused on the transcribed part of the gene that is predicted to code for protein and the intron sequence flanking the splice-sites of individual exons. The screens employed in this study depended on PCR amplification of genomic DNA and assumes that both alleles are represented equally in the amplified product. Heterogeneity at this locus may interfere with specific PCR primers annealing to specific alleles, preventing the amplification of one allele but not the other.

This report summarizes the clinical findings associated with eight OTOF mutations, five of them novel, in 65 NSRHL and NSRAN families. One of these families has an allele associated with a temperature-sensitive AN/AD phenotype. A temperature-sensitive allele for otoferlin should provide a valuable tool to understand the function of otoferlin and its role in the auditory process.

Acknowledgements

This grant was supported by the NIH Center for Hearing Loss in Children 5P60DC00982, the NIH NIDCD Program Project Grant 2P01DC0813-07 (WJK) and the Deafness Research
Foundation (PMK). This study was also supported in part by a grant from the NIDCD R01-DC02842 (RJHS), NIDCD R01-DC02618 (AS), The Oberkotter Foundation (LJH, CIB). Computing services were provided by the Genetic Sequence Analysis Facility at the University of Nebraska Medical Center and are supported by the Nebraska Research Initiative.

References


JMG/2005/038612/A potential OTOF temperature-sensitive allele


JMG/2005/038612/A potential OTOF temperature-sensitive allele


Figure Legend

Figure 1. Pedigrees of families with OTOF mutations. Proband is identified with arrow

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive license (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd to permit this article (if accepted) to be published in Journal of Medical Genetics and any other BMJPG products and sublicenses such use and exploit all subsidiary rights, as set out in our license (http://JMG.bmjournals.com/misc/fora/licenceform.shtml).
OTOF mutations revealed by genetic analysis of hearing loss families including a potential temperature-sensitive auditory neuropathy allele


J Med Genet published online December 21, 2005

Updated information and services can be found at:
http://jmg.bmj.com/content/early/2005/12/21/jmg.2005.038612.citation

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

Molecular genetics (1254)
Genetic screening / counselling (887)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/