TBC1D24 and non-syndromic autosomal dominant hearing loss: the identification of an additional Italo-American family carrying the p.(S178L) mutation

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Abstract

Hearing loss is the most common sensorineural disorder, affecting approximately 1:1000 new-borns. In developed countries, more than half of the cases of congenital hearing loss are due to genetic causes and both syndromic and non-syndromic forms may be recognized. Approximately 20% of the cases of non-syndromic hearing loss are inherited according to an autosomal dominant pattern. Autosomal dominant hereditary hearing loss (ADHHL) is characterized by a wide genetic heterogeneity and by inter- and intra-familial clinical variability, making genotype-phenotype correlations extremely complicated.

Here we describe a large multi-generation Italo-American kindred affected by ADHHL. After a complete clinical evaluation and hearing function assessment through pure tone audiometry, the proband underwent a multiple-step genetic testing. Eventually, whole exome sequencing was performed on his and other selected family members' DNA leading to the identification of a heterozygous missense variant in the *TBC1D24* gene. Mutations in this gene have been associated with a variety of conditions that are inherited in an autosomal recessive pattern and that may or may not include hearing loss. Interestingly, the variant identified in our kindred is the only mutation in the *TBC1D24* gene that has been associated with ADHHL in previous studies. Our case report confirms the role of the *TBC1D24* gene and specifically of the p.(S178L) variant in the etiopathogenesis of ADHHL, underlining once again the clinical variability associated with variants in this gene.

Keywords: Autosomal dominant hereditary hearing loss, TBC1D24, Whole Exome Sequencing

Introduction

Congenital hearing loss is the most frequent sensorineural disorder with a prevalence of 1:1000 new-borns (Korver et al., 2017). In developed countries, more than 50% of congenital hearing loss cases are due to genetic causes and Non-Syndromic Hereditary Hearing Loss (NSHHL) accounts for almost two-thirds of them (Cohn et al., 1999). Most cases of NSHHL are inherited in an autosomal recessive pattern, about 15-20% of them are autosomal dominant and a minority of cases are X-linked or mitochondrial (Grundfast et al., 1999, 1067–1088; Korver et al., 2017; Shearer et al., 1993).

Autosomal dominant hereditary hearing loss (ADHHL) usually is a post-lingual disorder, with onset between the second and the fifth decade of life. It often is progressive and may begin as a high-frequency hearing impairment later involving the middle and lower frequencies as well, with variable degree of severity (Petersen, 2002, 1–13). ADHHL is characterized by a huge inter- and intra-familial clinical variability, which implies that even from parent to child or between siblings the phenotype might display extensive inconsistencies, challenging the achievement of a correct clinical diagnosis and making

genotype-phenotype correlations extremely complicated (Vona et al., 2015, 260–270). So far, almost 50 different genes have been involved in ADHHL (Hearing Loss Homepage. https://hereditaryhearingloss.org) and some of them have been described only in single families, underlining how the identification of the molecular cause of the disease might be hampered by a substantial genetic heterogeneity (Gao et al., 2018, 298–306). In this light, the employment of cutting-edge genetic tests, such as Whole Exome Sequencing (WES), might help both in the discovery of novel genes related to this phenotype and in the determination of a genetic diagnosis for those families that still remain unsolved after multiple-step analyses.

Case report

Here we describe a large multi-generation Italo-American family affected by ADHHL recruited at the Institute for Maternal and Child Health - I.R.C.C.S. "Burlo Garofolo" (Trieste, Italy) (Figure 1). All genetic tests and clinical evaluations have been performed by the Medical Genetics Unit of the same hospital. All patients provided written informed consent and all research was conducted according to the ethical standard defined by the Helsinki Declaration.

The proband (V:24) firstly underwent a careful clinical examination and dysmorphology assessment, that did not outline any significant anomaly. Afterwards, hearing evaluation was performed on the proband and other affected family members (IV:1, IV:15, IV:23, V:11) by pure tone audiometry, which showed a bilateral, symmetric, and moderate to severe sensorineural hearing loss with a medium and high frequencies drop threshold profile (Figure 2). Tympanometry was also performed and showed a type A tympanogram for all the evaluated subjects; there was no evidence of vestibular dysfunction in any of the assessed patients. The age of onset of the hearing impairment ranged from the second to the third decade of life and the disease was slowly progressive over time.

The proband (V:24) underwent a multiple-step genetic testing which included the analysis of mutations in *GJB2* and *GJB6* genes and in 96 additional genes associated with NSHHL (Vozzi et al., 2014, 209-216). All tests resulted negative therefore WES using Illumina NextSeq 550 sequencer (Illumina, San Diego, CA, USA) was performed on the proband and four other selected members of the family, both affected and healthy (IV:1, IV:15, V:11, V.17). The analysis led to the identification in the affected individuals of a heterozygous missense variant in the *TBC1D24* gene (NM_001199107.1, c.533C>T, p.(S178L)). Sanger sequencing (Figure 3A) was performed on additional 32 family members both affected and healthy (IV generation: subject 23; V generation: subjects 3, 8, 9, 13, 15, 19, 21, 26, 27, 28, and 36; VI generation: subjects 3, 5, 6, 7, 8, 9, 10, 11, 14, 15, 16, 17, 18, 19, 21, 22, 23, and 25; VI generation: subjects 1, and 2), confirming the correct segregation of the variant within affected individuals.

Results

The *TBC1D24* gene encodes a member of the Tre2-Bub2-Cdc16 (TBC) domain-containing RAB-specific GTPase-activating proteins that is called TBC1 domain family member 24. Two main domains may be recognized in the protein: the TBC domain, which may serve as a GTPase-activating domain, and the TLDc domain that is believed to be involved in oxidative stress resistance (Falace et al., 2010, 365–370; Finelli & Oliver, 2017, 395–406). This protein is thought to have a role in neuronal projections development and is involved in the regulation of membrane and synaptic vesicle trafficking (Falace et al., 2010, 365–370; Lüthy et al., 2019, 2319–2335). Previous studies have shown how TBC1 domain family member 24 is almost ubiquitously expressed in mouse, with the highest expression detected in the brain, especially during cortical development, followed by testis, skeletal muscle, heart, kidney, lung, and liver (Falace et al., 2010, 365–370). Further studies have highlighted how this protein is expressed in mouse cochlea, being localized in the stereocilia of the inner and outer hair cells and in the spiral ganglion neurons (Zhang et al., 2014, 814–818).

Mutations in this gene have been associated with several diseases, both syndromic and non-syndromic, with an autosomal recessive pattern of inheritance. They comprise differ-

ent forms of epilepsy, including familial infantile myoclonic epilepsy, Rolandic epilepsy with paroxysmal exercise-induced dystonia and writer's cramp, and developmental and epileptic encephalopathy 16 (MIM#: 605021; MIM#: 608105; MIM#: 615338). Moreover, mutations in the *TBC1D24* gene have been associated with DOORS syndrome (MIM#: 220500), a complex disorder characterized by deafness, onychodystrophy, osteodystrophy, intellectual disability, and seizures. Additionally, biallelic mutations in this gene have been associated with NSHHL, specifically autosomal recessive deafness 86 (MIM#: 614617).

Most notably, according to literature data, the heterozygous missense variant c.533C>T, p.(S178L) identified in our kindred is the only mutation in the *TBC1D24* gene that has been associated with ADHHL (Deafness, autosomal dominant 65 - MIM#: 616044) (Azaiez et al., 2014, 819–823; Zhang et al., 2014, 814–818) (Figure 3B). In the previously described families the audiometry pattern is consistent with the one detected in our kindred, with individuals displaying as well a non-syndromic, bilateral, slowly progressive hearing impairment that involved initially the high frequencies and that gradually progressed to all frequencies, reaching a severe-to-profound severity in the oldest affected subjects.

The c.533C>T variant causes a change in the highly conserved Serine-178 residue within the TBC domain of the protein (Figure 3C), substituting serine, which is a polar amino acid, with leucine, that is hydrophobic. This substitution may alter the correct folding process of the protein, its stability, or its proper function. Whereas biallelic loss of function mutations might be considered the main pathogenic mechanism for the autosomal recessive forms of *TBC1D24*-related hearing loss, it may be hypothesised that this missense variant could cause ADHHL through a dominant-negative mechanism, specifically since it is located in the GTPase-activating domain. Nevertheless, little is still known about this protein function, therefore further functional studies are required to unravel this hypothesis.

Conclusion

The identification of the c.533C>T, p.(S178L) variant in the *TBC1D24* gene through WES in our large kindred underlines how this technique might be extremely helpful in the determination of a genetic diagnosis for those cases that still remain unsolved after a multiple-step approach analysis. Furthermore, our case report confirms the role of the *TBC1D24* gene in the etiopathogenesis of ADHHL, endorsing the peculiarity of this variant in causing the phenotype. The function of TBC1 domain family member 24 is still widely unknown and further studies are necessary both to define the exact role of this protein in brain and ear development and to characterize the molecular mechanisms through which this variant causes hearing impairment. In conclusion, the available literature data and our case report highlight the wide variability of clinical phenotypes associated with *TBC1D24* variants, underlining how a precise genotype-phenotype correlation might sometimes be extremely challenging.

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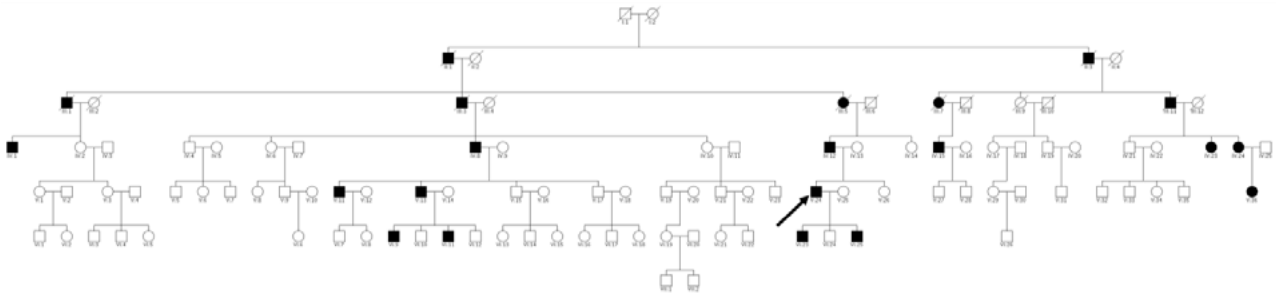
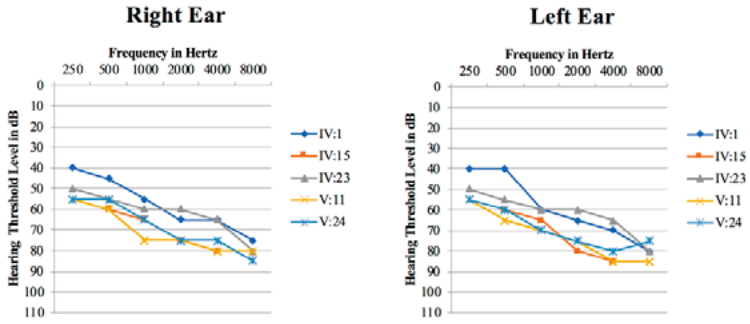
Figures

Figure 1. Pedigree of the Italo-American family.



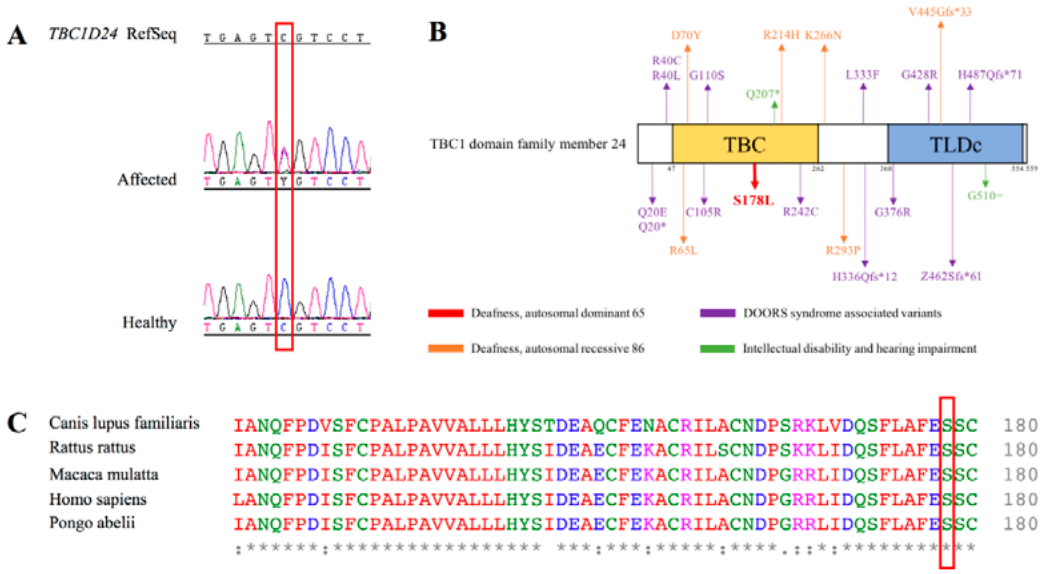
Pedigree of the family investigated in the present study. Filled symbols represent affected individuals. The proband is indicated with an arrow.

Figure 2. Audiograms of the Italo-American family.



Audiometric features of the proband and four additional family members are displayed as audiograms (air conduction). The thresholds of the right and left ears are shown.

Figure 3. DNA chromatograms, distribution of *TBC1D24* mutations, and protein alignment.



A *DNA sequence chromatograms showing the TBC1D24 nucleotide variant identified in the family.*
B *Graphic representation of TBC1 domain family member 24 and the location of the pathogenic variants associated with hearing loss. Variants are grouped by colour on the basis of the clinical phenotype. According to the Human Gene Mutation Database (HGMD) professional database the splicing mutation c.1206+5G>A has been associated with DOORS syndrome, nevertheless it has not been included in the picture since the effect on the protein level has not been reported (Campeau et al., 2014, 44–58). TBC= Tre2-Bub2-Cdc16 domain; TLDc= Tre2/Bub2/Cdc16 (TBC), lysin motif (LysM), catalytic domain.*
C *Protein alignment showing conservation of residue S178 across species.*