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# Unresolved questions regarding human hereditary deafness

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## Abstract

Human hearing loss is a common neurosensory disorder about which many basic research and clinically relevant questions are unresolved. This review on hereditary deafness focuses on three examples considered at first glance to be uncomplicated, however, upon inspection, are enigmatic and ripe for future research efforts. The three examples of clinical and genetic complexities are drawn from studies of (1) Pendred syndrome/*DFNB4* (PDS, OMIM 274600), (2) Perrault syndrome (deafness and infertility) due to mutations of *CLPP* (PRTLS3, OMIM 614129), and (3) the unexplained extensive clinical variability associated with *TBC1D24* mutations. At present, it is unknown how different mutations of *TBC1D24* cause nonsyndromic deafness (DFNB86, OMIM 614617), epilepsy (OMIM 605021), epilepsy with deafness, or DOORS syndrome (OMIM 220500) that is characterized by deafness, onychodystrophy (alteration of toenail or fingernail morphology), osteodystrophy (defective development of bone), intellectual disability and seizures. A comprehensive understanding of the multifaceted roles of each gene associated with human deafness is expected to provide future opportunities for restoration as well as preservation of normal hearing.

### Keywords

hereditary deafness; enlarged vestibular aqueduct; *SLC26A4*; Pendred syndrome; Perrault syndrome; *CLPP*; *TBC1D24*

## Introduction and Background Information

For every 1000 neonates, one or two will have a permanent, significant hearing loss (Morton & Nance, 2006). Throughout adolescence, the prevalence of hearing loss markedly increases. A majority of eighty-year-olds has a variety of deficits including compromised

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ability to detect and discriminate between sounds, referred to as presbycusis. At any age, the foremost reasons for hearing loss are genetic and environmental factors. Acquired hearing loss can be caused by loud noise, cytomegalovirus infection and ototoxic drugs such as cisplatin and aminoglycoside antibiotics (Brock et al., 2012, Barbi et al., 2003). Environmental factors in combination with a specific genotype may cause deafness as well. As an example of a gene-environment interaction collectively causing deafness, the likelihood that head trauma or barotrauma will precipitate hearing loss is increased in subjects carrying mutations of the *SLC26A4* gene (Usami et al., 1999). Similarly, approximately 15% of persons in the United States with aminoglycoside-associated hearing loss have the m.1555A>G mutation in their mitochondrial *MTRNR1* gene, which is transmitted matrilineally and encodes the mitochondrial 12S ribosomal RNA (Prezant et al., 1993, Fischel-Ghodsian et al., 1997). This is a challenging medical predicament because aminoglycoside antibiotics are often inexpensive, widely available and sometimes a clinical necessity.

## Nonsyndromic deafness

Genetic deafness is classified by the mode of inheritance of the mutation causing deafness and whether or not hearing loss is accompanied by other clinical relevant disorders as part of a syndrome. Approximately 60% to 70% of cases of inherited nonsyndromic deafness (i.e. absence of co-segregating extra-auditory features) are associated with autosomal recessive mutations (referred to as DFNB), 20% to 30% are autosomal dominant (DFNA), and a few percent are located on the X-chromosome (referred to as DFNX or X-linked). There are now approximately 100 different nonsyndromic deafness genes that have been reported (Griffith & Friedman, 2016). Some of the genes associated with human deafness have both dominant and recessive mutations. Examples include *TMC1* (Kurima et al., 2002), *MYO7A* (Liu et al., 1997) and the *TBC1D24* genes (Azaiez et al., 2014). Although there are nearly one hundred nonsyndromic deafness genes, less than 10 of them account for a majority of cases of nonsyndromic hearing loss inherited as a recessive trait (DFNB). Three of the most common genes underlying autosomal recessive nonsyndromic deafness are *GJB2* at the *DFNB1* locus on chromosome 13 (OMIM 220290), *SLC26A4* at the *DFNB4* locus on chromosome 7q22.3 (OMIM 600791) and *MYO15A* at the *DFNB3* locus on chromosome 17p11.2 (OMIM 600316). Usually, but not always, the genotype of a deafness gene can accurately predict the phenotype. Some deafness-causing mutations resist the influence of environmental factors or modifiers in the genetic background, and thus are said to be fully penetrant. Although difficult to study and not yet well understood, there are examples of protective variants in our genetic backgrounds that can influence the severity and age of onset of hearing loss (Riazuddin et al., 2000, Bykhovskaya et al., 2000, Schultz et al., 2005).

Depending on the ethnicity, a particular pathogenic mutation may be a common cause of deafness. For example, in southern Europe, New Zealand, Australia and North America, a deletion of one G nucleotide (c.35delG) in *GJB2* encoding connexin 26 accounts for an estimated 50% of recessive nonsyndromic deafness (Smith & Van Camp, 1993–2016). Among Ashkenazi Jews, the recessive c.167delT mutation of *GJB2* is carried by approximately 4% of individuals (95 percent confidence interval, 2.5 to 6.0%) and explains a majority of nonsyndromic deafness in this population (Morell et al., 1998).

Is a genetic variant benign or pathogenic? Every human genome is assumed to have numerous mutations whose pathogenic potential is difficult to predict. An average human is heterozygous for approximately 100 loss-of-function recessive mutations and is homozygous for approximately 20 loss-of-function alleles (MacArthur et al., 2014, Tennessen et al., 2012). It is not uncommon to detect these mutations as coincidental variants in wide-scale DNA sequencing. Global encyclopedias of human genomic variation facilitate the interpretation of many variants as pathogenic or coincidental. Some variants initially considered to cause deafness have eventually been recognized as non-pathogenic. For example, variants of *MYO1A* encoding myosin 1A were reported to cause deafness but were subsequently found to be benign polymorphisms (Abou Tayoun et al., 2015, Donaudy et al., 2003, Eisenberger et al., 2014).

Genetic testing for many of the genes unequivocally demonstrated to be associated with prelingual (before the acquisition of speech)-onset deafness is commercially available and may establish a molecular diagnosis of childhood hearing loss. However, there are caveats. Notwithstanding a genotype predicted to cause a pathogenic phenotype, there are instances of nonpenetrance (i.e. nonappearance of the expected mutant phenotype) (Chen et al., 2016). Moreover, siblings may have a seemingly identical phenotype but the deafness may be caused by mutations of different genes (Rehman et al., 2015). When a molecular genetic cause of deafness is known in one sibling, it is not necessarily a safe assumption that the same genotype is responsible for deafness of a sibling or deaf relative (Rehman et al., 2015).

## Syndromic deafness

Approximately one third of individuals with an inherited hearing loss also have additional abnormalities of other tissues or organs, which is referred to as syndromic deafness. There are at least 400 clinically distinct forms of syndromic deafness annotated in the Online Mendelian Inheritance in Man (http://www.omim.org). Different mutations of a single gene may be associated with nonsyndromic or syndromic hearing loss. For example, mutations of *PCDH15* encoding protocadherin 15 can cause either type 1 Usher syndrome characterized by congenital profound deafness, vestibular areflexia and progressive loss of vision due to retinitis pigmentosa, or just nonsyndromic deafness DFNB23 (Ahmed et al., 2003, Ahmed et al., 2001). Similarly, mutations of *CDH23* encoding cadherin 23 can also cause a type 1 Usher syndrome or nonsyndromic deafness DFNB12 (Bolz et al., 2001, Bork et al., 2001). Although the number of subjects was small, when a person had an Usher syndrome-associated mutation of one *CDH23* allele in combination with a nonsyndromic deafness-associated allele of CDH23 (referred to as a compound heterozygosity), the resultant phenotype was nonsyndromic deafness DFNB12 (Schultz et al., 2011). These data indicate that a DFNB12-associated mutant *CDH23* allele, hypothesized to have a low level of residual function, is phenotypically dominant to an USH1D (Usher syndrome type 1D, OMIM 601067)-associated mutant CDH23 allele. Perhaps a low level of CDH23 function is sufficient in the eye to maintain vision but is insufficient in the sensory epithelial cells of the inner ear to preserve hearing (Schultz et al., 2011). These observations have implications for therapies for retinitis pigmentosa in Usher syndrome. If only a small amount of partially functional PCDH15 and CDH23 protein is actually necessary to maintain vision, a therapy

for Usher syndrome to maintain vision need not aim to achieve a normal level of these proteins to restore or preserve vision.

## Pendred Syndrome, enlarged vestibular aqueduct and the mystery of missing mutations

Pendred syndrome (OMIM 274600) is inherited as an autosomal recessive disorder characterized by bilateral severe to profound prelingual- or perilingual-onset sensorineural hearing loss, enlargement of the vestibular aqueduct (EVA, OMIM 600791) and incompletely penetrant goiter with a typical onset around the time of sexual maturity (Phelps et al., 1998, Morgans & Trotter, 1958, Fraser, 1965). Pendred syndrome is caused by mutations of the *SLC26A4* gene on chromosome 7q31 (Everett et al., 1997). SLC26A4, also called pendrin, is a transmembrane protein capable of ATP-independent exchange of chloride, iodide, bicarbonate and other anions. To date, *SLC26A4* is the only gene with mutations reported to cause Pendred syndrome. Mutations of *SLC26A4* were also reported to cause nonsyndromic deafness DFNB4 (Li et al., 1998). Different ethnic groups have their own distinct spectrum of mutations of *SLC26A4*. Depending on the population, Pendred syndrome accounts for as much as 10% of hereditary deafness and 7.5% of childhood deafness (Park et al., 2003, Fraser, 1965).

Most patients with two recessive mutations (biallelic mutations) of *SLC26A4* develop Pendred syndrome although they initially present with nonsyndromic hearing loss and EVA. The most penetrant manifestation of Pendred syndrome is EVA. Computed tomography (CT) of the temporal bone reveals enlargement of the vestibular aqueduct while magnetic resonance imaging (MRI) shows enlargement of the soft tissue and fluid contents of the vestibular aqueduct: the endolymphatic duct and sac.

Only 25% of European or North American Caucasian individuals with EVA have two mutant alleles of *SLC26A4.* Fifty percent of subjects with EVA have no obvious mutations in the transcribed region of the *SLC26A4* gene and 25% have only one mutant allele of *SLC26A4*. It has been hypothesized that EVA may result from digenic inheritance of one recessive *SLC26A4* mutation and a second recessive mutation in another gene (Yang et al., 2009, Yang et al., 2007), but the evidence is not conclusive (Choi et al., 2009). Variants of *KCNJ10* and *FOXI1* have been associated with EVA although support for the involvement of these genes is not robust (Abou Tayoun et al., 2015, Yang et al., 2009, Yang et al., 2007) and has not been reproducible.

Other possibilities to explain only one or no mutant alleles in or near the exons of *SLC26A4* in many EVA subjects include unidentified alterations of the regulatory sequences that remotely control *SLC26A4* gene expression. However, efforts to identify such mutations have heretofore been unsuccessful. We note that enhancers of a gene need not even be linked to chromosome 7q31 (Kleinjan & van Heyningen, 2005, van Heyningen & Bickmore, 2013). An innovative experimental strategy will be required to identify the missing variants associated with the increased risk of EVA.

In mice, a null mutation (no functional SLC26A4 protein) of the *Slc26a4* gene causes enlargement of endolymph-containing spaces, loss of the endocochlear potential, deafness, and vestibular dysfunction (Everett et al., 1997). There is also a "tet-on" mouse model where the only expression of SLC26A4 is from an *Slc26a4* transgene introduced onto a *Slc26a4* null genetic background. In the tet-on mouse model, induction of expression of *Slc26a4* from a transgene requires the presence of doxycycline in drinking water (Choi et al., 2011). Data from this study indicated that *Slc26a4* expression is required during development from embryonic day16.5 to postnatal day 2 for the initial acquisition of normal hearing at one month after birth (Choi et al., 2011). However, a low level of SLC26A4 expression is necessary to maintain normal hearing in the *Slc26a4* "tet-on" mouse model (Choi et al., 2011, Li et al., 2013). This mouse model of EVA can be used to identify and test potential therapeutic options to prevent progression or fluctuation of hearing loss that is often associated with EVA in young children.

## Perrault Syndrome and unresolved questions about male fertility

Perrault syndrome (PS, OMIM 233400) is a clinically and genetically heterogeneous sex-influenced rare recessive disorder that is characterized by sensorineural hearing loss of variable severity in men and women and ovarian disorders ranging from nonfunctional streak ovaries to premature ovarian insufficiency before the age of 40 years (Newman et al., 1993–2016, Jenkinson et al., 2013). As teenagers, young women with a normal karyotype and affected with PS may be hypoestrogenic and have an elevated level of follicle stimulating hormone and lutenizing hormone mimicking menopause (reviewed in Newman et al., 2014). Hypergonadotropic hypogonadism is a characteristic hormone profile of women with PS. Occasionally, other clinically relevant neurological signs have also been noted to co-occur and include ataxia, nystagmus, learning disability and progressive peripheral neuropathy (Pierce et al., 2010, Demain et al., 2016).

Recessive mutations of five genes are now known to be associated with PS, with many more PS genes remaining to be discovered (Demain et al., 2016, Jenkinson et al., 2012, Jenkinson et al., 2013, Pierce et al., 2011, Pierce et al., 2010). In one family, PS is associated with compound heterozygosity for two missense mutations of *HARS2*, encoding mitochondrial histidyl tRNA synthetase. These data suggested that PS is predominantly a disorder of mitochondrial proteostasis (Pierce et al., 2011). In support of this interpretation, a homozygous missense mutation of *LARS2* was identified in a family with one deaf female and her two deaf brothers. Additionally, PS can also be caused by mutations of *LARS2*, encoding mitochondrial leucyl-tRNA synthetase (Pierce et al., 2013, Solda et al., 2015) and mutations of *CLPP* (Jenkinson et al., 2013). *CLPP* encodes a highly conserved chambered mitochondrial protease. Why are manifestations of dysfunctional mitochondrial proteostasis due to recessive mutations of *CLPP*, *HARS2* and *LARS2* limited largely to ears and ovaries? An answer to this question may emerge from investigations of animal models of PS mutations to explore the pathogenic mechanisms of hearing loss and ovarian impairment (Gispert et al., 2013).

In addition to *CLPP*, *LARS2* and *HARS2,* mutations of *HSD17B4* are associated with PS. *HSD17B4* encodes a multifunctional peroxisomal pre-protein that is proteolytically cleaved

into two separate enzymes, a 17-β-hydroxysteroid dehydrogenase type 4 and a hydratase, each catalyzing successive steps in fatty acid β-oxidation. Two hearing-impaired sisters were reported to be compound heterozygous for alleles of *HSD17B4* and exhibited ovarian dysgenesis, short stature, compromised cognitive ability and peripheral neuropathy (Fiumara et al., 2004, McCarthy & Opitz, 1985, Pierce et al., 2010). More disabling mutations of *HSD17B4* cause D-Bifunctional Protein deficiency (DBP deficiency; OMIM 261515). DBP deficiency is characterized by, but not limited to, perinatal hypotonia, seizures, craniofacial dysmorphology, hearing loss, vision impairment and a failure to thrive (Ferdinandusse et al., 2006, Lieber et al., 2014). Missense mutations of *C10ORF2* encoding TWINKLE have recently extended the spectrum of mutant genes associated with PS (Morino et al., 2014, Demain et al., 2016).

Reduced fertility in PS women is documented comprehensively but there is much less data on the fecundity of deaf males with biallelic mutations of the PS genes. Consequently, there are limited data to support the assumption that deaf males have normal fertility when they have the same mutant genotype as a woman with PS. In a single family reported with a mutation of *HARS2*, there were three PS females and their two deaf male siblings (Pierce et al., 2011), one of whom was reported 32 years ago to have normal hearing children (Pallister & Opitz, 1979, Pierce et al., 2011). For the other PS genes, caution is suggested in assuming normal fertility in deaf males with PS genotypes for the *LARS*, *HSD17B4* and *CLPP* genes. This matter is not resolved for three reasons. First, there are only a few deaf male siblings in all families reported to be segregating PS and fertility in only one male has been documented (Pallister & Opitz, 1979, Pierce et al., 2011). The scarcity of males in “Perrault families” may reflect an ascertainment bias towards families with deaf women with primary amenorrhea and a 46, XX karyotype (Newman et al., 1993–2016). Second, clinical data on sperm count and sperm motility for deaf males in Perrault families have not been reported. Third, and most vexing, a homozygous recessive null allele of *Clpp* in mouse causes deafness and sterility of both females and males (Gispert et al., 2013). A caveat is that in mouse a complete knockout of the *Clpp* gene does not precisely recapitulate the two human *CLPP* missense mutations associated with PS, which may have reduced, but not absent, proteolytic function in mitochondria (Jenkinson et al., 2013). Gene editing with TALENs or CRISPR/Cas9 system could be used to engineer the equivalent of the human PS p.Cys147Ser, p.Thr145Pro, and p.Cys144Arg missense mutations into the mouse *Clpp* gene (Demain et al., 2016, Jenkinson et al., 2013).

## *TBC1D24* Mutations Associated with Nonsyndromic Deafness, Epilepsy or DOORS

There is no shortage of human genes associated with deafness whose normal functions are not well understood. One such example is *TBC1D24* located on chromosome 16p that belongs to a family of TBC (Tre-2, Bub2, Cdc16) domain containing RAB-specific GTPase-activating proteins. The eight annotated exons of the *TBC1D24* (NM_001199107) encode a 559-amino acid protein that has an N-terminal TBC domain and a C-terminal TLDc domain (TBC, LysM, domain catalytic). Smaller isoforms of *TBC1D24* are also expressed (see supplemental figure S1; (Rehman et al., 2014)). Some TLDc domain-containing proteins

have a neuro-protective role against oxidative stress (Oliver et al., 2011, Durand et al., 2007, Finelli et al., 2016). *TBC1D24* is expressed in various tissues including inner ear, brain, kidney, heart and liver. In the inner ear, TBC1D24 protein is expressed in the spiral ganglion neurons (Rehman et al., 2014) and also in the inner and outer hair cells (Azaiez et al., 2014). The question remains as to whether or not a normal function, or an additional normal function, of TBC1D24 is to diminish oxidative stress within spiral ganglions and in other inner ear cells types where it is expressed.

There are now 26 reported mutant alleles of *TBC1D24* that are distributed across the gene, some of which are located in the sequences that encode the TBC and TLDc domains (Figure 1). Mutations of *TBC1D24* are associated with nonsyndromic deafness segregating as an autosomal recessive trait (DFNB86, OMIM 614617) or as an autosomal dominant trait (DFNA65, OMIM 616044), familial infantile myoclonic epilepsy (FIME, OMIM 605021) with or without deafness, early infantile epileptic encephalopathy (EIEE16, OMIM 615338), progressive myoclonic epilepsy (PME, OMIM 310370), or deafness, onychodystrophy, osteodystrophy, mental retardation and seizures (DOORS, OMIM 220500). It is a conundrum as to how different homozygous or compound heterozygous mutations of *TBC1D24* can give rise to such different phenotypes.

As illustrated in Figure 1, seven of 26 mutations of *TBC1D24* are associated with nonsyndromic deafness (i.e no additional clinical anomalies) (Ali et al., 2012, Azaiez et al., 2014, Bakhchane et al., 2015, Rehman et al., 2014, Zhang et al., 2014). There are also ten mutant alleles of *TBC1D24* reported in patients with epileptic syndromes (Corbett et al., 2010, Falace et al., 2010, Afawi et al., 2013, Guven & Tolun, 2013, Milh et al., 2013, Doummar et al., 2015, Muona et al., 2015, Poulat et al., 2015, Strazisar et al., 2015). With these observations in mind, two of the four available DFNB86 families segregating the nonsyndromic deafness-associated mutation p.Asp70Tyr of *TBC1D24* were re-examined to determine if the subjects had epilepsy. A detailed family history of epilepsy was collected from 15 affected and 18 unaffected individuals from the two families. Magnetic resonance imaging (MRI) of the brain and electroencephalography (EEG) was also performed on selected affected and unaffected individuals. A history of seizures was reported in one of the 18 normal-hearing individuals. An 18-year-old deaf female had fever-associated seizures at the age of 8 years and reports no seizures since then. The remaining 17 normal-hearing individuals and 14 deaf individuals have never experienced a seizure. Given the high prevalence of epilepsy, co-occurrence of fever-associated seizures with hearing loss in one of the 15 deaf individuals could be coincidental (Rehman et al., 2014).

The association of *TBC1D24* with nonsyndromic deafness was further solidified by identification of three missense and one frameshift mutation segregating in three Moroccan families (Bakhchane et al., 2015). Affected individuals from three families displayed severe to profound congenital sensorineural deafness and were compound heterozygous for two mutant alleles of *TBC1D24*. All deaf individuals shared the p.Arg214His allele and one of the following three variants as the second allele; p.Glu153Lys, p.Lys266Asn, or p.Val445Glyfs*33. The p.Arg214His mutation has an allele frequency of 2% in a presumably normal-hearing Moroccan control population. To date, no deaf individuals homozygous for p.Arg214His have been reported. One possible explanation is that

p.Arg214His is a hypomorphic allele (residual normal function) and individuals homozygous for p.Arg214His have no obvious loss of hearing. The p.Arg214His may be associated with hearing loss only in compound heterozygosity with a more disabling mutation of *TBC1D24*.

With the exception of p.Ser178Leu, all other reported mutations of *TBC1D24* are recessive. In a family of European descent and also in a Chinese family, p.Ser178Leu co-segregated with nonsyndromic dominant progressive hearing loss (Azaiez et al., 2014, Zhang et al., 2014). Individuals who are heterozygous (carriers) for truncating recessive mutations of *TBC1D24* are phenotypically normal (Bakhchane et al., 2015, Guven & Tolun, 2013, Milh et al., 2013, Poulat et al., 2015). Therefore, the p.Ser178Leu allele is likely to cause hearing loss either through a gain-of-function or a dominant-negative mechanism and not due to haplo-insufficiency for TBC1D24 function (Zhang et al., 2014).

Recessive mutations of *TBC1D24* are also associated with a range of epileptic disorders with or without hearing loss (Figure 1) or with DOORS syndrome. The diverse epileptic phenotypes include familial infantile myoclonic epilepsy without intellectual impairment, progressive myoclonus epilepsies, focal epilepsy with intellectual disability, early onset epileptic encephalopathy with hearing loss, and malignant migrating partial seizures of infancy (Corbett et al., 2010, Doummar et al., 2015, Falace et al., 2010, Guven & Tolun, 2013, Milh et al., 2013, Poulat et al., 2015, Strazisar et al., 2015, Muona et al., 2015). DOORS syndrome segregates as an autosomal recessive trait and presents extensive clinical variability. DOORS patients who have mutations of *TBC1D24* exhibit five consistent features; (1) deafness, (2) intellectual disability or developmental delay, (3) seizures, (4) small distal phalanges, and (5) small or absent nails (Campeau et al., 2014a, Campeau et al., 2014b). In addition to these five main clinical features of DOORS, some patients have optic and peripheral neuropathy, visual impairment, triphalangeal thumbs, facial dysmorphisms and microcephaly. A cohort of 38 patients from 32 families segregating DOORS syndrome was screened to identify the underlying genetic basis. This effort led to identification of 10 recessive mutations of *TBC1D24* in thirteen patients from 11 families. There are at least 23 DOORS syndrome patients with no mutations in *TBC1D24* suggesting that there are other genes in which mutations can cause DOORS syndrome. Alternatively, there are unidentified variants associated with DOORS located in the regulatory elements of *TBC1D24*.

The location and type of mutations of *TBC1D24* cannot currently be used to predict the associated phenotype. However, the same mutant allele in different individuals appears to cause a similar phenotype suggesting a minor role for genetic modifiers. The two exceptions to such a simplistic generalization are the mutations p.Glu153Lys and p.His336Glnfs*12. Homozygosity for the p.Glu153Lys allele results in familial infantile myoclonic epilepsy with moderate intellectual disability but, in compound heterozygosity with p.Arg214His, causes nonsyndromic deafness (Bakhchane et al., 2015, Poulat et al., 2015). Similarly, the p.His336Glnfs*12 allele in compound heterozygosity with an experimentally demonstrated splice site mutation, c.1206+5G>A, causes DOORS syndrome. In contrast, p.His336Glnfs*12 in compound heterozygosity with the missense allele p.Asp11Gly results in epilepsy, hearing loss, and developmental delay, but no onychodystrophy or osteodystrophy (Strazisar et al., 2015). As no obvious genotype-phenotype relationship has

emerged for mutations of *TBC1D24*, predicting the phenotype associated with novel mutations of *TBC1D24* is not likely to be straightforward. Identifying the spectrum of protein partners that interact with TBC1D24, studying the phenotypes of engineered mutant mouse models of *Tbc1d24*, or studying the physical structure of TBC1D24 protein in relationship to the locations of amino acid substitutions might reveal a conceptual framework for the correlation of *TBC1D24* genotype and phenotype.

## Conclusions

Additional genes associated with nonsyndromic and syndromic forms of hearing loss remain to be discovered. With time, molecular genetic screening will eventually include all of the deafness-causing genes as well as the *cis*-acting sequences necessary for regulating their expression. Improvements in genomic sequencing and its interpretation will continue to expand the benefits of genetic testing available for patients. These benefits include estimates of recurrence probability in a family, reducing misconceptions about the biological reason for hearing loss, and identification of deafness-associated medical conditions that require surveillance or management, and hopefully in the future, guidance for precision therapies.

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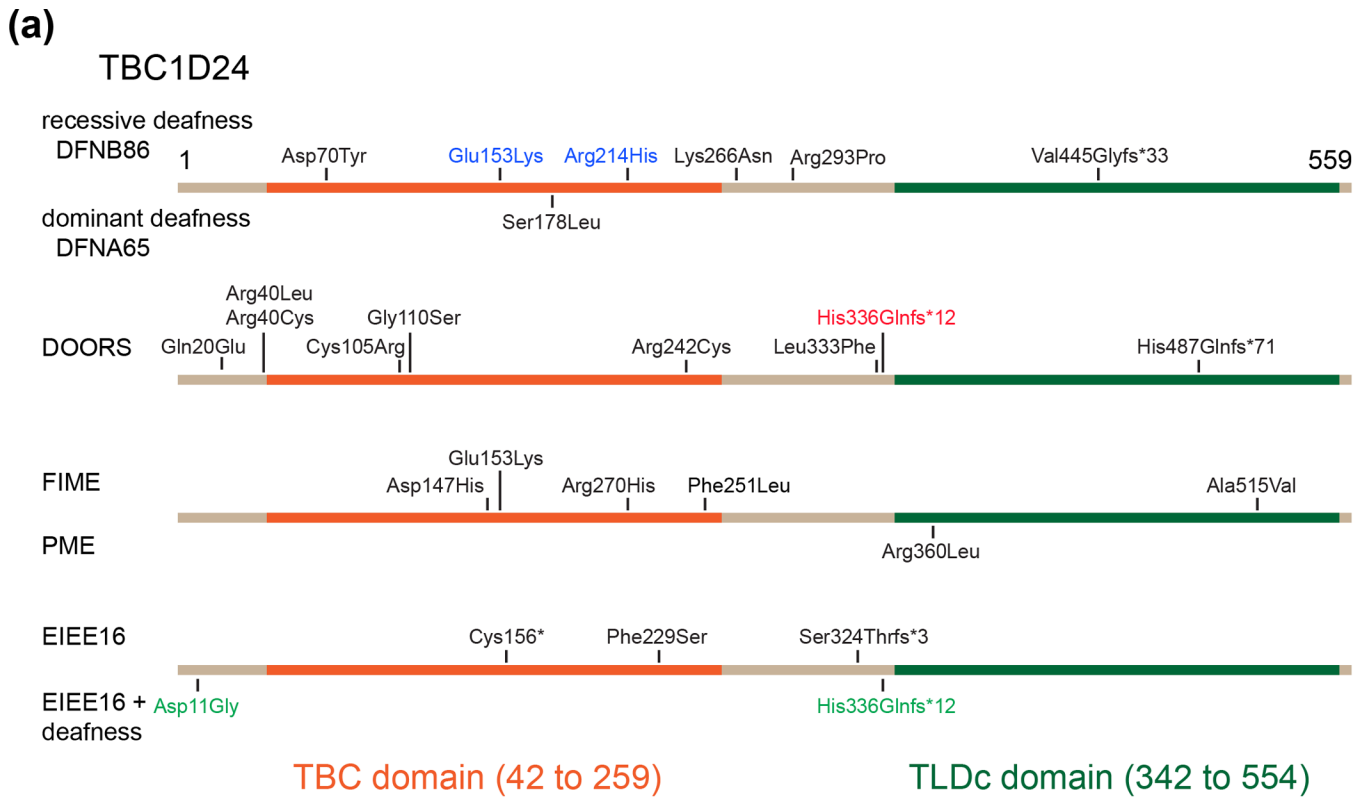



**Figure 1.**

Neurological disorders caused by mutations of *TBC1D24*. **(a)** Each distinct disorder is caused by different subset of twenty-six pathogenic mutations of *TBC1D24*, with two exceptions (p.Glu153Lys and p.His336Glnfs*12 shown in blue and red font, respectively). The p.Glu153Lys in homozygosity results in FIME (myoclonic epilepsy, familial infantile, OMIM 605021) while in compound heterozygosity with p.Arg214His causes nonsyndromic deafness DFNB86 (OMIM 614617). The p.Asp11Gly in compound heterozygosity with p.His336Glnfs*12 causes EIEE (epileptic encephalopathy, early infantile, 16 OMIM 615338) and deafness whereas in compound heterozygosity with an experimentally confirmed splice site mutation c.1206+5G>A causes DOORS syndrome (deafness, onychodystrophy, osteodystrophy, mental retardation and seizures, OMIM 220500). Mutation nomenclature is based on the full-length isoform of *TBC1D24* (NM_001199107.1). The c.1333dupG(p.Val445Glyfs*33) was reported as c.1316insG(p.Val439Glyfs*32) in Bakhchane et al., 2015 based on a shorter isoform of *TBC1D24* (NM_020705, personal communication with the last author). **(b)** *TBC1D24* gene structure and location of a splice site mutation c.1206+5G>A that, in compound heterozygosity with p.His336Glnfs*12, causes DOORS syndrome. Thick and thin bars

represent exons and introns, respectively. PME; progressive myoclonic epilepsy (OMIM 310370).