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SLC26A4 Genotypes and Phenotypes Associated with Enlargement of the Vestibular Aqueduct

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Key Words

SLC26A4 • Pendred syndrome • Hearing • Deafness • Inner ear • Genotype-phenotype correlation

Abstract

Enlargement of the vestibular aqueduct (EVA) is the most common inner ear anomaly detected in ears of children with sensorineural hearing loss. Pendred syndrome (PS) is an autosomal recessive disorder characterized by bilateral sensorineural hearing loss with EVA and an iodine organification defect that can lead to thyroid goiter. Pendred syndrome is caused by mutations of the *SLC26A4* gene. *SLC26A4* mutations may also be identified in some patients with nonsyndromic EVA (NSEVA). The presence of two mutant alleles of *SLC26A4* is correlated with bilateral EVA and Pendred syndrome, whereas unilateral EVA and NSEVA are correlated with one (M1) or zero (M0) mutant alleles of *SLC26A4*. Thyroid gland enlargement (goiter) appears to be primarily dependent on the presence of two mutant alleles of *SLC26A4* in pediatric patients, but not in older patients. In M1 families, EVA may be associated with a second, undetected *SLC26A4* mutation or epigenetic modifications. In M0 families, there is probably etiologic heterogeneity that includes causes other than, or in addition to, monogenic inheritance.

Introduction

Approximately one in 1000 children are born with hearing loss [1]. Morton [2] estimated that half of all cases of hearing loss with an onset prior to the normal development of speech and language (i.e. prelingual onset) are thought to have a hereditary basis. Most hereditary hearing loss is sensorineural (SNHL) and inherited as a simple Mendelian trait. Hearing loss is described as nonsyndromic or syndromic, respectively, according to the absence or presence of abnormalities of other organs, such as the kidney, heart, or eye [3]. Seventy percent or more of hereditary hearing loss cases appear to be nonsyndromic [4]. Syndromic

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hearing loss accounts for the remaining 30% of genetic SNHL phenotypes in children, with a growing list of hundreds of hearing loss syndromes and genes [5]. Nonsyndromic SNHL also has a high degree of genetic heterogeneity with more than 160 loci and more than 60 causative genes that have been identified to date (Van Camp G, Smith RJH. Hereditary Hearing Loss Homepage, Accessed August 11, 2011 at http://hereditaryhearingloss.org). There is overlap among these groups of genes because some of them underlie both syndromic and nonsyndromic hearing loss phenotypes [6]. Discovery of a causative gene for a syndromic hearing loss phenotype is often followed by identification of less severe or nonsyndromic phenotypes associated with mutant alleles of the same gene. This extension of a phenotypic spectrum can also occur with nonsyndromic hearing loss disorders in which information from gene expression studies or mouse models can guide later detection of subtle syndromic findings in patients initially diagnosed with nonsyndromic hearing loss.

Pendred syndrome

The diagnostic criteria for Pendred syndrome have evolved since 1896 when Vaughan Pendred first reported the combination of goiter and hearing loss in two sisters [7]. Their severe hearing losses had been present since infancy, while goiters were first noted at the age of 13 years. In 1927, Brain [8] described 12 individuals who had profound hearing loss since birth and developed goiter in childhood. He postulated that both phenotypes were associated with a common hereditary factor and inherited as a recessive trait. The biochemical defect underlying goiter in Pendred syndrome was later shown to be a thyroidal iodine organification defect [9]. This defect affecting thyroid hormone synthesis could be detected by measuring the discharge of a radioactive inorganic iodide isotope from the thyroid after administration of potassium perchlorate. Potassium perchlorate is a competitive inhibitor of the sodium-iodide symporter, which transports iodide into thyroid folliculocytes across their basolateral membrane. An abnormally high discharge of iodide from the thyroid gland in response to perchlorate administration, in combination with congenital deafness, became the gold standard for the clinical diagnosis of Pendred syndrome [10].

Fraser [11] defined Pendred syndrome as congenital deafness, goiter and a positive perchlorate discharge test and estimated its prevalence at 7.5 to 10 per 100,000 people. This indicated that Pendred syndrome might account for up to 10% of hereditary hearing loss [11]. Advances in radiographic imaging of the temporal bones led to the proposal that a Mondini malformation of the cochlea should be included as an essential feature of the diagnosis [12, 13]. Indeed, histological studies of Pendred syndrome temporal bones revealed an incomplete bony partition of adjacent turns of the cochlea (Mondini malformation), enlargement of the vestibular aqueduct (EVA), and a variety of membranous labyrinth abnormalities [14]. A subsequent report revealed similar findings in four additional patients with Pendred syndrome [15], while another study concluded that the Mondini defect was the underlying cause of SNHL in Pendred syndrome [16].

SLC26A4

The causative gene for Pendred syndrome was identified as *SLC26A4* (originally called *PDS*) in 1997 [17]. The identification of *SLC26A4* led to re-evaluation of the original family used to define nonsyndromic recessive deafness DFNB4, which was linked to the same region on chromosome 7q31 [18]. The affected members of the original DFNB4 family were re-diagnosed with Pendred syndrome [19]. Most clinicians now rely upon molecular testing of *SLC26A4* for the diagnosis of Pendred syndrome. There are over 160 reported mutations in *SLC26A4*, which has been associated with sporadic and familial forms of Pendred syndrome in populations worldwide. Furthermore, a large-scale study demonstrated mutations of *SLC26A4* in approximately 5-10% of childhood SNHL patients among a variety of different ethnic populations [20], providing a genotypic confirmation of Fraser's phenotypic estimate of the prevalence of Pendred syndrome [11].

SLC26A4 encodes an 86-kDa transmembrane protein named pendrin with up to 15 predicted membrane-spanning domains [17, 21]. Mouse *Slc26a4* is expressed in a restricted tissue distribution that includes the inner ear, thyroid, kidney, lung, and several other organs [17]. Pendrin has been shown to exchange anions and bases across the plasma membrane in several heterologous expression systems. It is thought to mediate Cl^-/I^- exchange in the thyroid [22] and Cl^-/HCO_3^- exchange in the inner ear [23]. This anion exchange activity appears to be critical during late embryonic and early postnatal development of the inner ear

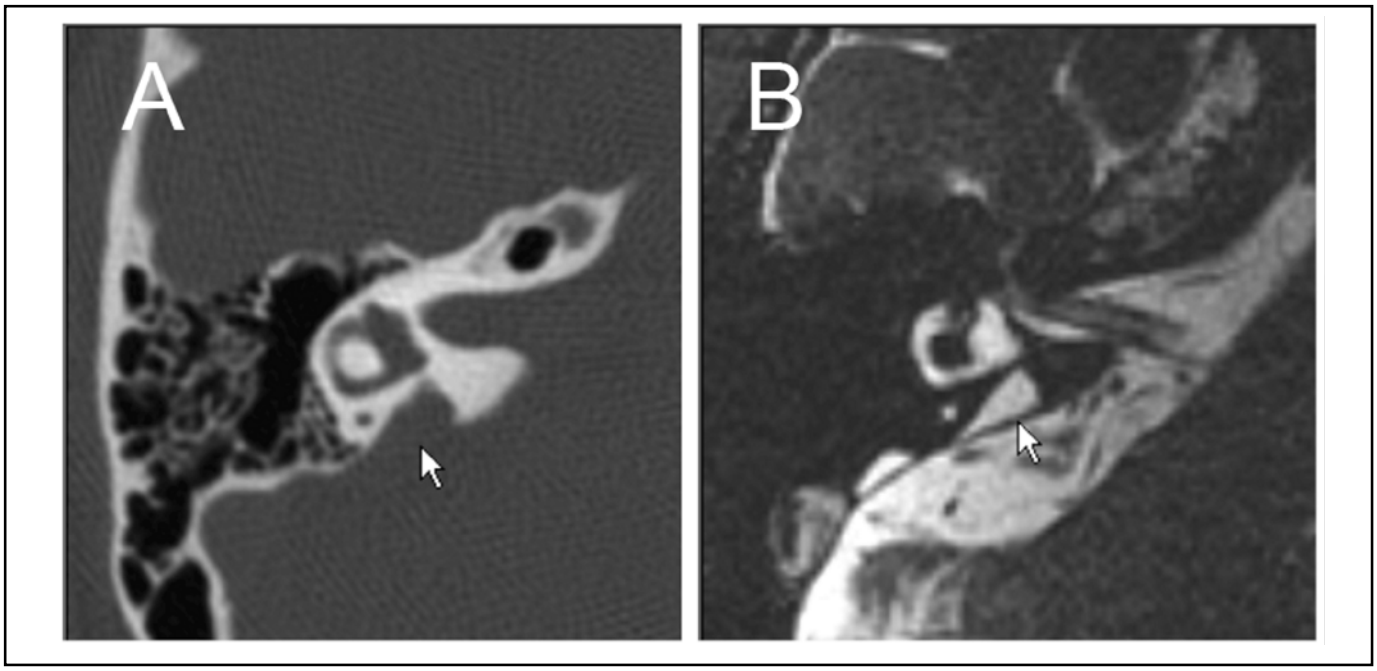

Fig. 1. Radiologic imaging of a right temporal bone with an enlarged vestibular aqueduct. (A) Axial computed tomography (CT) scan of an enlarged vestibular aqueduct (arrow). (B) Axial magnetic resonance (MR) image of the same ear showing an enlarged endolymphatic sac and duct (arrow). Reproduced from http://www.nidcd.nih.gov/health/hearing/eva-intro.htm.

[24]. The molecular details of this pathogenetic mechanism remain to be fully elucidated [25, 26].

Enlargement of the vestibular aqueduct (EVA)

Modern radiologic imaging of temporal bones and molecular testing for *SLC26A4* mutations established that the Mondini cochlear malformation was neither a consistent nor specific observation in Pendred syndrome. Enlargement of the vestibular aqueduct (EVA) is now recognized as the most penetrant feature of Pendred syndrome [27]. EVA was initially defined as a vestibular aqueduct diameter greater than or equal to 1.5 mm measured midway between the operculum and the common crus on computed tomography (CT) scans (Fig. 1A) [28]. Magnetic resonance (MR) imaging reveals enlargement of the endolymphatic sac and duct, which courses through the vestibular aqueduct (Fig. 1B) [27]. The relationship of the vestibular aqueduct and endolymphatic duct and sac is shown in Fig. 2.

EVA is also identified in patients with nonsyndromic hearing loss. Griffith et al. reported a family in which two brothers had SNHL and EVA without syndromic abnormalities. Their hearing loss and EVA was postulated to be inherited as a recessive trait [29]. Other authors reported additional familial cases [30] described as 'nonsyndromic hearing loss with EVA (NSEVA)'. Molecular genetic testing revealed that many cases of NSEVA are associated with *SLC26A4* mutations. First, a large Asian Indian family was reported to segregate EVA, without goiter, with homozygosity for a mutant allele of *SLC26A4* at the DFNB4 locus [31]. Usami et al. identified *SLC26A4* mutations in sporadic and familial cases of NSEVA, indicating that *SLC26A4* mutations are commonly associated with NSEVA [32]. Their observations were confirmed in numerous studies of large cohorts of NSEVA patients from different ethnic populations [33-37].

EVA has also been detected in a subset of patients with branchio-oto-renal (BOR) or branchio-oto (BO) syndrome [38], Waardenburg syndrome [39], and deafness associated with the recessive form of distal renal tubular acidosis [40]. There is no published evidence that mutations of the genes underlying these syndromes cause NSEVA.

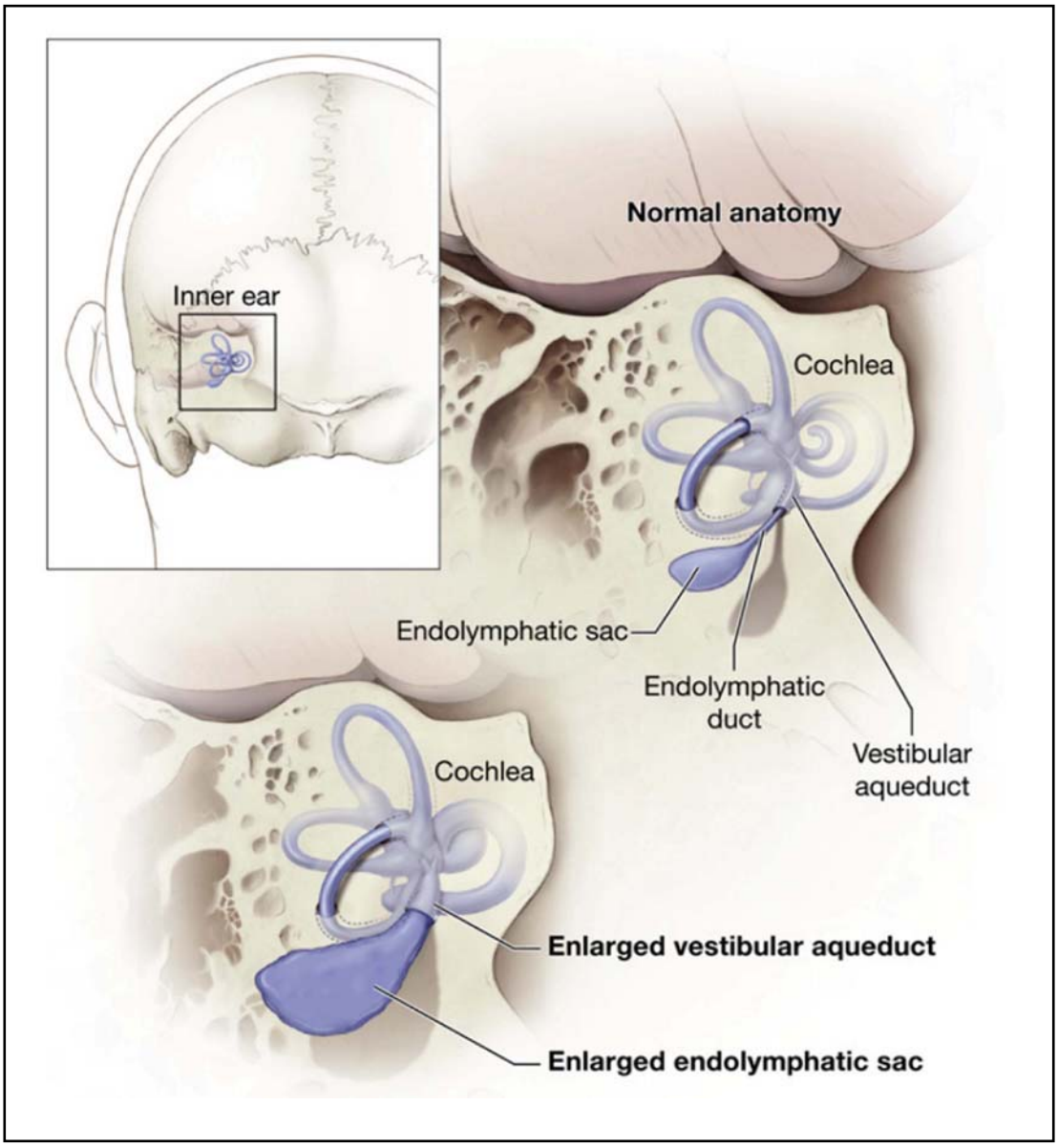

Fig. 2. Schematic illustration of an enlarged vestibular aqueduct and endolymphatic sac and duct. Reproduced from http://www.nidcd.nih.gov/health/hearing/vestAque.htm.

SLC26A4 genotype-phenotype correlation

The detection of *SLC26A4* mutations both in Pendred syndrome and NSEVA has led to efforts to identify potential genotype-phenotype correlations. Scott et al. [33] used an anion transport assay in *Xenopus* oocytes to ask whether *SLC26A4* mutations detected in patients with Pendred syndrome differed functionally from *SLC26A4* mutations detected in individuals with NSEVA. They concluded that hypo-functional pendrin encoded by NSEVA alleles had residual transport activity that prevents the development of goiter, while functional null alleles led to Pendred syndrome [33]. However, this correlation was not consistent with later studies that identified null alleles in NSEVA and hypo-functional alleles in Pendred syndrome [34, 37, 41]. There are some variants that are detected in NSEVA but not Pendred syndrome, but many of these variants are probably nonpathogenic and coincidentally detected [42]. Taken together, the current published reports do not support a correlation between the type of *SLC26A4* mutation and thyroid phenotype (i.e. NSEVA versus PS).

In North American and European EVA populations, *SLC26A4* mutations cannot be detected in up to one half of patients, while only one mutant *SLC26A4* allele is identified in one fourth of patients [35-37, 43]. We refer to the detection of only one or zero mutant alleles of *SLC26A4* as non-diagnostic because two mutant alleles are required to cause an autosomal recessive trait

[44]. The proportion of EVA patients with non-diagnostic *SLC26A4* genotypes is higher when the radiologic criterion is changed to include vestibular aqueducts with midpoint diameters as small as 1.0 mm [45, 46]. Pryor et al. examined the potential correlation of clinical phenotype and the number of mutated alleles of *SLC26A4* in patients with EVA. They defined Pendred syndrome as EVA and >15% discharge of iodide two to three hours after administration of perchlorate. Pendred syndrome was correlated with the presence of two mutant alleles of *SLC26A4* (M2), while NSEVA was associated with either one (M1) or zero (M0) mutant alleles of *SLC26A4* [35]. In addition, unilateral EVA was correlated with only one or zero mutant allele of *SLC26A4*, while two mutant alleles of *SLC26A4* was tightly correlated with bilateral EVA. The number of mutant alleles of *SLC26A4* was thus strongly associated with the thyroid (perchlorate discharge test result) and auditory phenotypes. The perchlorate discharge phenotype-genotype correlation was dependent upon uniform test procedures and interpretation, and comprehensive screening to exclude patients with other confounding phenotypic abnormalities [35]. Madeo et al. [47] used ultrasonography to calculate thyroid gland volume and defined goiter by comparison to published normative data. Thyroid gland enlargement was primarily dependent on the presence of two mutant alleles of *SLC26A4* in pediatric (<10 years old) EVA patients, but not in older EVA patients [47].

Etiology of EVA in patients with non-diagnostic *SLC26A4* genotypes

In order to understand the causes and recurrence risk of EVA in families of probands with non-diagnostic *SLC26A4* genotypes (M1 or M0), Choi et al. evaluated the segregation ratio of EVA among siblings in M2, M1 and M0 families [44]. A segregation ratio is the proportion of offspring that can be expected to be of a particular genotype or phenotype. The segregation ratios of EVA in M1 and M2 families were not statistically different from 0.25, which is expected for an autosomal recessive trait with full penetrance and viability. However, the segregation ratio was significantly lower among M0 families. Furthermore, *SLC26A4*-linked polymorphic DNA markers co-segregated with EVA in M1, but not M0 families. These results were consistent with the existence of a second, undetected *SLC26A4* mutation that accounts for EVA in the M1 families [44]. However, nucleotide sequence analyses of conserved noncoding regions in and around the *SLC26A4* gene failed to identify occult mutations, and comparative genome hybridization (CGH)-microarray analysis similarly failed to identify pathogenic copy number variants at the *SLC26A4* locus [44]. These analyses could have failed to detect copy number variations affecting short segments of DNA, or mutations in intronic or regulatory regions that were not analyzed. Alternatively, epigenetic modifications of *SLC26A4* such as DNA methylation might repress transcription [48] and account for the observed co-segregation of EVA and *SLC26A4* in M1 families. The correlation of less severe, nonsyndromic EVA phenotypes with M1 genotypes may reflect undetected mutant or epigenetically-modified alleles of *SLC26A4* that exert their effects as hypomorphs with residual function [44], in a tissue (inner ear)- or time-specific manner [24], or a combination of these mechanisms. It is unlikely that a single mutant allele of *SLC26A4* is sufficient to cause EVA since there are no published reports of vertical co-segregation of EVA with a single mutant allele of *SLC26A4* or of sporadic cases associated with a single *de novo* mutant allele of *SLC26A4* [35].

In M0 families, the low segregation ratio and discordant inheritance of *SLC26A4*-linked DNA markers with EVA suggested etiologic heterogeneity that includes causes other than, or in addition to, monogenic Mendelian inheritance [44]. Congenital cytomegalovirus (CMV) infection is a very common cause of childhood SNHL and can produce an auditory phenotype that is very similar to that of EVA [49]. However, congenital CMV infection was ruled out as a common or significant cause of EVA [50].

In M1 families, a single pathogenic mutation of *SLC26A4* might cause EVA in combination with a mutation in another gene [35]. Yang et al. described several EVA patients with a heterozygous *SLC26A4* mutation in combination with a heterozygous hypo-functional variant of *FOXI1* [51] or *KCNJ10* [52]. However, these results have not been obtained in other studies of EVA cohorts [53-55] and the pathogenic potential of the reported *FOXI1* and *KCNJ10* variants remains uncertain [56, 57].

The causes of EVA in patients with nondiagnostic *SLC26A4* genotypes are thus largely unknown. Identification of additional genes or factors underlying EVA would facilitate more precise diagnosis and could provide mechanistic insight into the pathogenesis of hearing loss in this enigmatic disorder.

Conclusions

EVA is the most common radiologic anomaly of the inner ear recognized in children with early-onset SNHL.

EVA is most commonly identified in either of two different phenotypic contexts, Pendred syndrome or NSEVA. Pendred syndrome and most cases of NSEVA are allelic disorders associated with mutations of *SLC26A4*.

There is no correlation between the type of *SLC26A4* mutation and thyroid phenotype (i.e. Pendred syndrome versus NSEVA), but the number of mutant alleles of *SLC26A4* is correlated with the thyroid and auditory phenotypes.

In M1 families, EVA is likely caused by a second, undetected *SLC26A4* mutation, while in M0 families, etiologic heterogeneity includes causes other than, or in addition to, monogenic inheritance.

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