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Genotype-phenotype correlations for *SLC26A4*-related deafness

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Abstract

Pendred syndrome (PS) and non syndromic enlarged vestibular aqueduct (EVA) are two recessive disorders characterized by the association of sensorineural hearing loss (SNHL) with inner ear malformations that range from isolated EVA to Mondini Dysplasia, a complex malformation that includes a cochlear dysplasia and EVA. Mutations in the *SLC26A4* gene, coding for the protein pendrin, have been implicated in the pathophysiology of both disorders. In order to determine whether *SLC26A4* genotypes can be correlated to the complexity and severity of the phenotypes, we ascertained 1506 deaf patients. Inner ear abnormalities were present in 474 patients (32%). Mutation screening of *SLC26A4* detected two mutations in 16% of patients, one mutation in 19% of patients and zero mutation in 65% of patients. When the distribution of *SLC26A4* genotypes was compared across phenotypes, a statistically significant difference was found between PS patients and non syndromic EVA-Mondini patients ($p = 0.005$), as well as between EVA patients and Mondini patients ($p = 0.0003$). There was a correlation between phenotypic complexity of inner ear malformations and genetic heterogeneity - PS patients have the most severe phenotype and the most homogeneous etiology while EVA patients have the least severe phenotype and the most heterogeneous etiology. For all patients, variability in the degree of hearing loss is seen across genotypes implicating other genetic and/or environmental factors in the pathogenesis of the PS-Mondini-EVA disease spectrum.

Keywords

Pendred Syndrome; EVA; Mondini dysplasia; *SLC26A4*; Genotype-Phenotype correlation

Introduction

Pendred syndrome (PS) is characterized by congenital sensorineural hearing loss (SNHL) and goiter. It is considered to be one of the most common forms of syndromic deafness and is estimated to account for about 10% of all hereditary hearing impairment (Park

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et al. 2003). It is an autosomal-recessive disorder caused by mutations in the *SLC26A4* gene located on chromosome 7q22-q31 (Everett et al. 1997). Over 90 different *SLC26A4* mutations have been reported, with each ethnic population having its own distinctive mutant allele series including a few prevalent founder mutations (Campbell et al. 2001; Lopez-Bigas et al. 2002; Park et al. 2003; Tsukamoto et al. 2003; Van Hauwe et al. 1998). Overall, mutations in *SLC26A4* may account for 5–10% of prelingual hearing loss (Reardon et al. 1997). The reported mutations are located throughout the coding region and include missense, nonsense, splice site and frameshift mutations, as well as a large partial genomic deletion (Park et al. 2003). Pendrin, the protein product of *SLC26A4*, is an anion transporter (Scott and Karniski 2000; Scott et al. 2000; Scott et al. 1999), expressed in the thyroid, kidney and inner ear. Defects in this protein result in neuroepithelial damage and inner ear malformations (Everett et al. 1999).

The clinical presentation of persons with mutations in *SLC26A4* is highly variable. Features range from classical PS (goiter and prelingual profound SNHL) to non-syndromic recessive hearing loss associated with enlargement of the vestibular aqueduct (EVA) in the absence of goiter (DFNB4). The clinical distinction between PS and DFNB4 can be difficult to make because the goiter phenotype is variably penetrant and is not usually evident until adolescence (Fraser 1965). The most common inner ear abnormality in PS is EVA, which is present in up to 80% of patients (Cremers et al. 1998; Reardon et al. 2000). In this study, we present a comprehensive analysis of 474 patients with temporal bone anomalies to determine whether *SLC26A4* genotypic and phenotypic data can be correlated.

Materials and methods

Patient Recruitment

Fifteen hundred and six persons with congenital deafness were sequentially accrued from hearing loss referrals to the Molecular Otolaryngology Research Laboratories, specifically excluding individuals with syndromic (other than PS), acquired or dominant types of hearing loss. Evaluation included a complete history and physical examination, audiometry and high-resolution computed tomography (CT) of the temporal bones. Genetic testing for *SLC26A4* mutations was performed for all patients with inner ear malformations. Informed consent to allow genetic testing was obtained from all participants or from the parents of minors. The University of Iowa Human Subjects Committee approved all procedures.

Clinical Evaluation

All patients underwent otoscopic examination and audiometric testing. In most cases, hearing levels were determined by pure-tone audiometry, which was completed using a diagnostic audiometer in a soundproof room, in accordance with International Standards Organization (ISO 8253-1-3) standards (Preciado and de la Guardia Brin 1986). In all patients, the presence of malformations of the cochlea and vestibular aqueduct was evaluated by CT scan of the temporal bones. To diagnose EVA, the vestibular aqueduct had to be greater in diameter than 1.5 mm at a point midway between the endolymphatic sac and the vestibule. To diagnose Mondini dysplasia, the cochlea had to show incomplete partition and a scala communis. The clinical diagnosis of PS was based on characteristic

clinical features including hearing loss, inner ear malformations and a palpable goiter or abnormal perchlorate discharge test. To compare hearing levels, the binaural mean PTA for air conduction at 0.5, 1, and 2 kHz ($PTA_{0.5,1,2kHz}$) was calculated. Average thresholds in the range of 21–40 dB were defined as mild hearing impairment (HI), in the range of 41–70 dB as moderate HI, in the range of 71–95 dB as severe HI, and >95 dB as profound HI (Smith et al. 2005). Thyroid ultrasound was performed to determine the presence or absence of goiter.

SLC26A4 Mutation screening

Denaturing high-performance liquid chromatography (DHPLC) and bidirectional DNA sequencing were used for mutation analysis of *SLC26A4* (Prasad et al. 2004) in 474 patients. PCR was used to detect del(*GJB6-D13S1830*), del(*GJB6*-D13S1854) as described by del Castillo et al (del Castillo et al. 2005; del Castillo et al. 2002). We considered as pathologic all allele variants of *GJB2* listed as nonsyndromic HI mutations on the Connexin-Deafness Home Page (Ballana et al. 2007). In addition, we considered two variants of debatable pathogenicity, M34T and V37I, to be pathologic.

Statistical analysis

Chi-square testing was used to determine if there was a significant difference in the observed number of mutant *SLC26A4* alleles in the EVA, Mondini and PS groups. For multiplex families, only the oldest affected person was included in the statistical analysis to avoid possible bias. Chi-square testing was also used to determine if there was a difference in the degree of hearing loss in the groups with one or zero *SLC26A4* mutations versus the group with two mutations; and to compare the unilaterality/bilaterality of hearing loss in the unilateral/bilateral EVA and unilateral/bilateral Mondini groups.

Estimation of the frequency of occult mutations (maximum likelihood estimates)

Estimation of the fraction of hidden mutations as well as the proportion of cases not due to *SLC26A4* gene was performed as described previously (Kimberling 2005). In addition to providing a means of testing for missed mutations, calculating maximum likelihood estimates also provides a method to estimate and test for the presence of genetic heterogeneity in the absence of linkage data.

Results

A total of 1506 patients with SNHL in whom a CT scan of the temporal bones was available for review were ascertained. The majority of patients were Caucasians. Inner ear abnormalities were present in 474 patients (32%), including 243 females and 231 males ranging in age from 2 to 67 years old. Inner ear abnormalities were bilateral in 376 patients and unilateral in 98 patients. Three hundred forty nine patients had either unilateral (89) or bilateral (260) EVA, and 125 patients had Mondini dysplasia (116 bilateral and 9 unilateral) (Table 1). Twenty-two of 376 patients (6%) with bilateral temporal bone anomalies also had goiter or an abnormal perchlorate discharge test and were classified as having PS. Fourteen multiplex families were included; two were classified as having PS and 12 as having non syndromic EVA (5), Mondini dysplasia (6) or both (1 family in which 1 sibling has EVA and the other has Mondini).

Genetic testing for *SLC26A4* mutation was performed in 474 patients from 458 families. Two mutations were detected in 66 patients (14.5%), one mutation was found in 89 patients (19.5%) and no mutation could be found in the remaining 303 patients (66%). Of those having two mutations, 38 patients had EVA and 28 patients had Mondini dysplasia. Among patients with only one mutation, 47 had bilateral EVA, 14 had unilateral EVA, 26 had bilateral Mondini and 2 had unilateral Mondini. Of those with no detectable mutation in *SLC26A4*, 242 patients had EVA (80%) and 61 had Mondini dysplasia (20%) (Table 1). There was a significant difference in mutation distribution between EVA patients and Mondini patients, with the presence of mutations in *SLC26A4* more frequently associated with the Mondini phenotype ($p = 0.0003$) (Fig 1a). In addition, bilateral malformations were also more frequently associated with the Mondini phenotype group as compared with the EVA phenotype ($p = 6 \times 10^{-5}$) (Fig 2). There was no difference in mutation distribution when bilaterality versus unilaterality was compared ($p > 0.05$) (Fig 2). When the PS patient group was compared to the non syndromic EVA/Mondini group (438), the distribution of zero, one or two *SLC26A4* mutations in the PS group was significantly different ($p = 0.005$) (fig 1b) (Table 2).

Mutations of *SLC26A4* were associated with a wide spectrum of hearing loss phenotypes ranging from mild-to-profound unilateral or bilateral hearing loss. Hearing loss was bilateral in 20% of patients with unilateral EVA and in 36% of patients with bilateral EVA ($p = 1\times10^{-8}$). None of the nine patients with unilateral Mondini dysplasia had bilateral hearing loss, while 3% of patients with bilateral Mondini had unilateral hearing loss ($p= 1\times10^{-8}$) (Fig 2). The distribution in the degree of hearing loss in patients with CT scan anomalies and two *SLC26A4* mutations was no different from that in patients with one or zero mutations ($p > 0.05$) (Fig 3).

Maximum likelihood estimates predict that we have detected 60% of the *SLC26A4* mutations in the patient population we have studied, leaving 40% of *SLC26A4* mutations undetected. Mutations in *SLC26A4* are also predicted to explain only ~40% of the phenotypes observed, implying that further gene discovery is warranted. We estimate that ~98% of cases with only a single mutation are compound heterozygotes in which the second pathologic allele has not been detected; in 8.5% of cases negative for *SLC26A4* mutations, there are two disease-causing mutations in this gene that remain to be discovered.

Discussion

Genetic testing for *SLC26A4* mutations was performed in 474 patients with inner ear abnormalities. The prevalence of biallelic mutations in this cohort was 14.5% and prevalence of monoallelic mutations was 19.5%. This prevalence does not change even when only patients with bilateral inner ear abnormalities are considered, and as such, our data are not consistent with other studies of predominantly Caucasian patients, which typically report that patient distribution is about one-third for each genotype (Coyle et al. 1998; Pryor et al. 2005). However, Albert and colleagues studied 100 EVA families and found a two-mutation prevalence of 24% and a one-mutation prevalence of 16%, results that are similar to ours (Albert et al. 2006).

It is unlikely that the reported differences reflect screening methodology. The high prevalence of one and zero *SLC26A4* mutations in EVA-Mondini patients could reflect limitations in our screening technique, which fails to detect deletions and promoter/enhancer mutations of *SLC26A4*. This possibility is supported by our estimate that only 60% of mutations in *SLC26A4* have been detected in our patient cohort, however other groups also used similar screening methodologies. Of note, our study, which is the largest to date, includes the greatest number of simplex cases. The number of simplex cases may be noteworthy as a substantial number make be non-genetic and therefore unlikely to recur (Campbell et al. 2001).

In contrast to reports that PS is a genetically homogeneous disorder caused by biallelic mutations in *SLC26A4* (Pryor et al. 2005), the absence of any mutations in 5 multiplex families and the presence of only a single mutation in another multiplex family suggest that Mondini-PS is more appropriately classified as a complex heterogeneous disease that is not exclusively caused by mutations in *SLC26A4*. Consistent with this observation is work by Hulander and colleagues proposing that mutations in *FOXI1* should be considered as a possible genetic factor for PS since the mouse mutant homozygous for the targeted deletion of this gene lacks expression of pendrin in the inner ear (Hulander et al. 2003). A recent study by Yang et al confirmed this hypothesis by demonstrating mutations in *FOXI1* and also in the *SLC26A4* promoter element to which it binds in patients with the PS-Mondini-EVA disease spectrum (Yang et al. 2007). Their results support a novel dosage-dependent model for the molecular pathogenesis of PS and non-syndromic EVA that involves *SLC26A4* and its transcriptional regulation.

Studies of the relationship between *GJB2* mutations and temporal bone anomalies as resolved by CT imaging have generally been negative, (Abe et al. 1999; Azaiez and Smith 2007; Denoyelle et al. 1999; Yaeger et al. 2006) although Propst and colleagues reported a series in which 72% of patients with temporal bone anomalies also had mutations in *GJB2* (Propst et al. 2006).

We found the distribution of *SLC26A4* mutations in PS versus non-PS (EVA-Mondini) patients to be significantly different ($p = 0.005$), with PS patients more likely to have two mutations (Fig 1b). This difference suggests that even though PS is a genetically heterogeneous disease, the degree of heterogeneity is not as great as the degree of heterogeneity seen with non-syndromic EVA-Mondini. Closer analysis of EVA-Mondini patients also shows that the prevalence of two, one and zero *SLC26A4* mutations in the EVA patient group is significantly different than in the Mondini patient group, with two mutations more likely associated with Mondini dysplasia than with EVA ($p = 0.0003$) (Fig 1a). The absence of a significant difference in genotypes when unilaterality and bilaterality are compared may reflect limited sample size (there are only a few unilateral cases). However, the possibility that unilateral malformations are more likely due to non-genetic factors than bilateral malformations is supported by the significant difference in the frequency of unilateral malformations in the EVA group versus the Mondini group ($p = 0.00006$). Mondini dysplasia is more likely to be of a genetic etiology than is EVA, and thus the occurrence of environmental phenocopies is less likely. In aggregate, our data imply that

there are more causes of the relatively mild EVA phenotype than there are of the multi-organ (ear and thyroid) PS phenotype.

Our hypothesis that the PS-Mondini-EVA disease spectrum represents a complex genetic disorder with possible environmental modifiers is consistent with the variability in hearing loss seen with *SLC26A4* mutations among affected siblings in multiplex families, and the wide range of hearing loss (mild to profound) among sporadic cases (Table 2, Fig 2). Variability exists even in patients with two *SLC26A4* mutations. Furthermore, the presence of bilateral hearing loss in 20% of patients with unilateral EVA and a unilateral hearing loss in 36% of patients with bilateral EVA is proof that structural changes of the temporal bone are not invariably the cause of hearing loss (Fig 3) (Jackler and De La Cruz 1989; Sheykholeslami et al. 2004). As a correlate, these data mean that one proposed mechanism for hearing loss - reflux of hyperosmolar endolymph from the endolymphatic sac and duct into the cochlea - cannot be invariably true (Jackler and De La Cruz 1989), which may explain why endolymphatic sac shunt procedures do not improve hearing (Valvassori and Clemis 1978).

In conclusion, our study confirms that the PS-Mondini-EVA spectrum of inner ear anomalies represents a genetically heterogeneous group of diseases. We have shown that the degree of hearing loss does not correlate with the number of mutated *SLC26A4* alleles. Our data have important implications for genetic testing and counseling in patients with inner ear anomalies. First, in addition to screening the coding sequence of *SLC26A4*, its regulatory region and *FOXI1* (two exons) must be analyzed. Second, failure to find two mutations in *SLC26A4* does not rule out PS and the clinician should continue to follow thyroid function in these patients.

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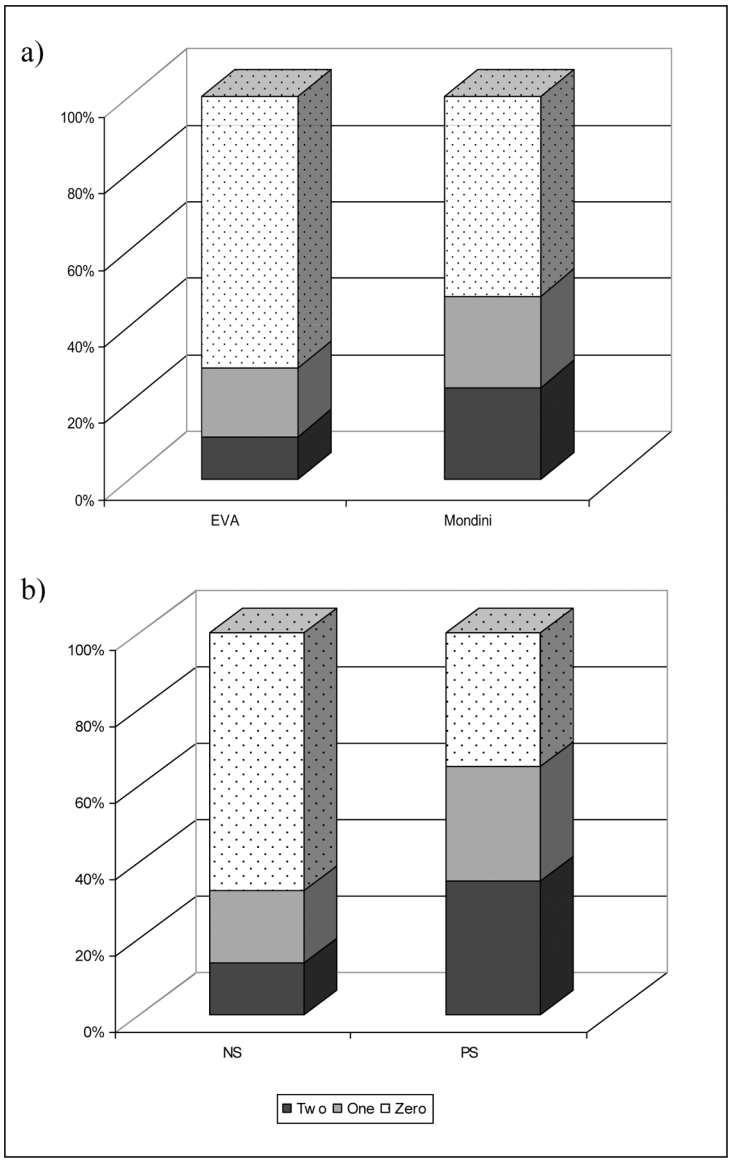

Figure 1:

Phenotype-genotype correlation for CT scan abnormalities and *SLC26A4* mutations. a). Distributions of number of mutated alleles among EVA and Mondini groups (Fisher's exact test p=0.0003). b) Distribution of the number of mutated alleles among PS and Non syndromic EVA-Mondini (NS) patients. Patients with PS are more likely to carry biallelic mutations. The proportion of zero mutation is much higher in the NS group (p=0.005).

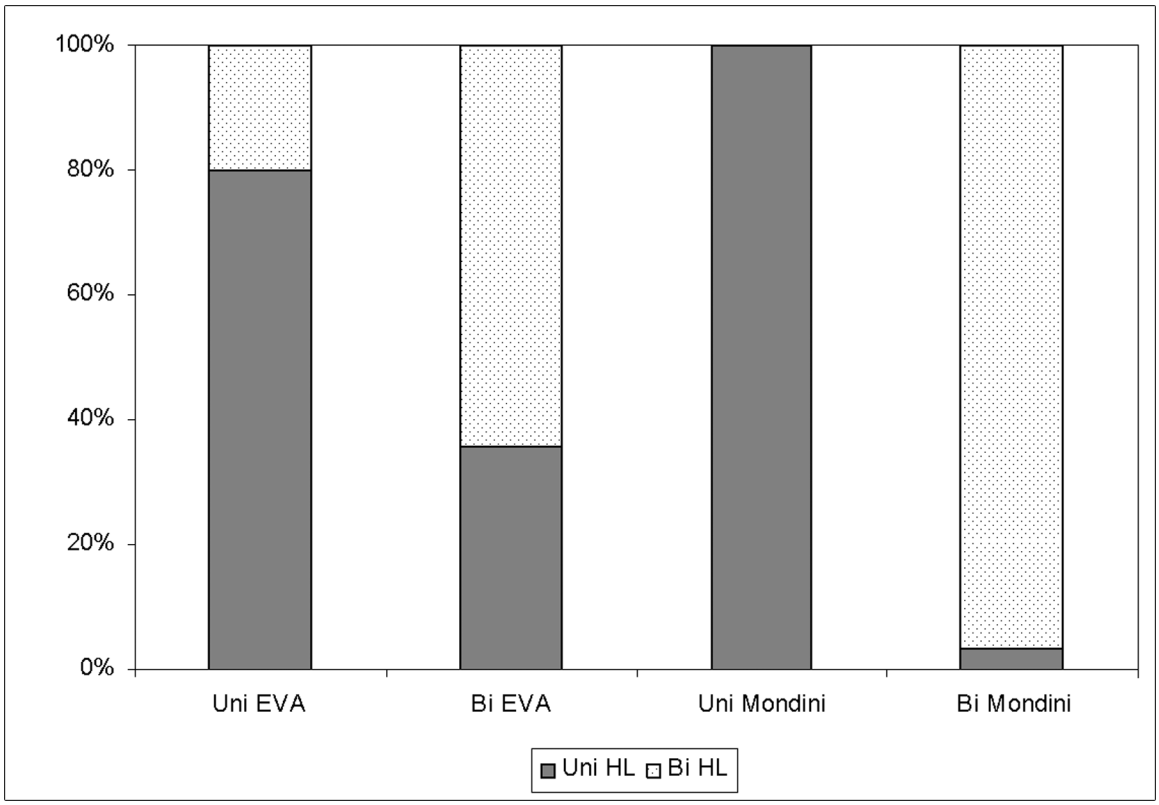

Figure 2:
Prevalence of unilateral and bilateral hearing loss in patients with unilateral or bilateral inner ear malformations. Bi: bilateral; Uni: unilateral, HL: hearing loss.

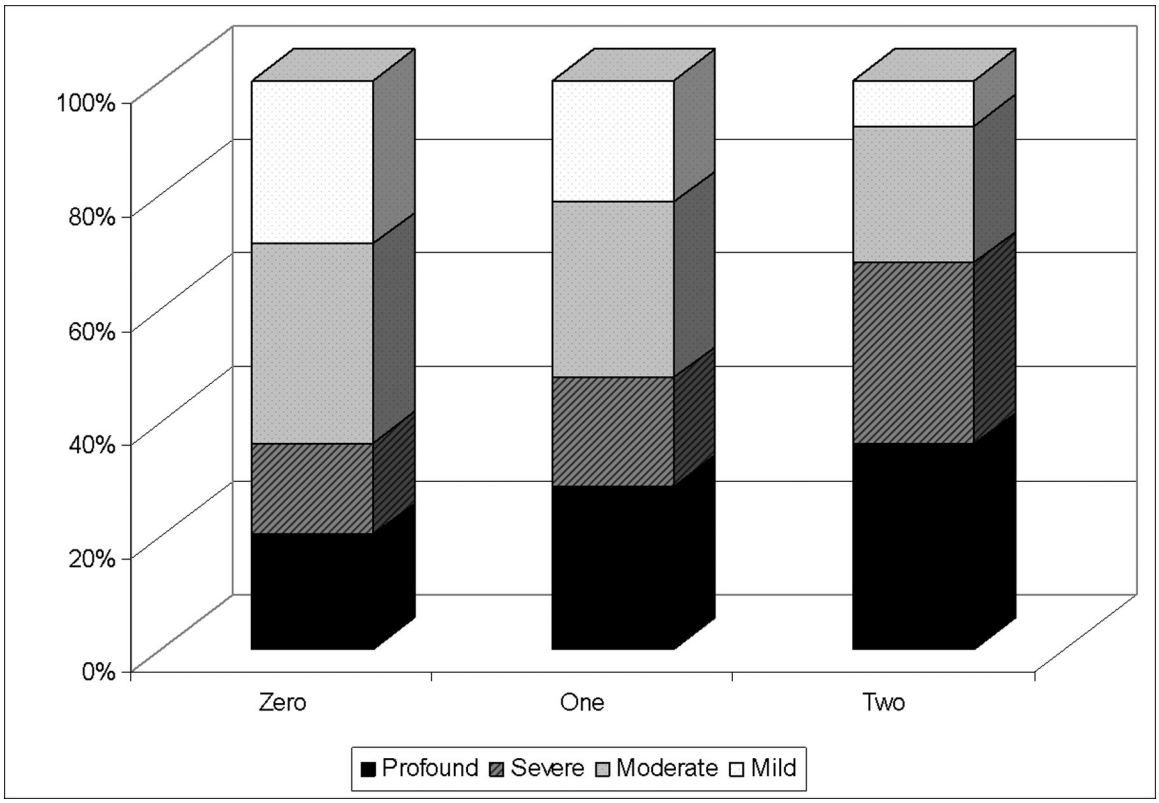

Figure 3:

Degree of hearing loss in patients with zero, one or two mutations. Mutations of *SLC26A4* were associated with a wide spectrum of hearing loss phenotypes. The degree of hearing loss distribution in patients with CT scan anomalies and two *SLC26A4* mutations is not statistically different from that in patients with only one or zero mutation. (Fisher's exact test $p>0.05$)

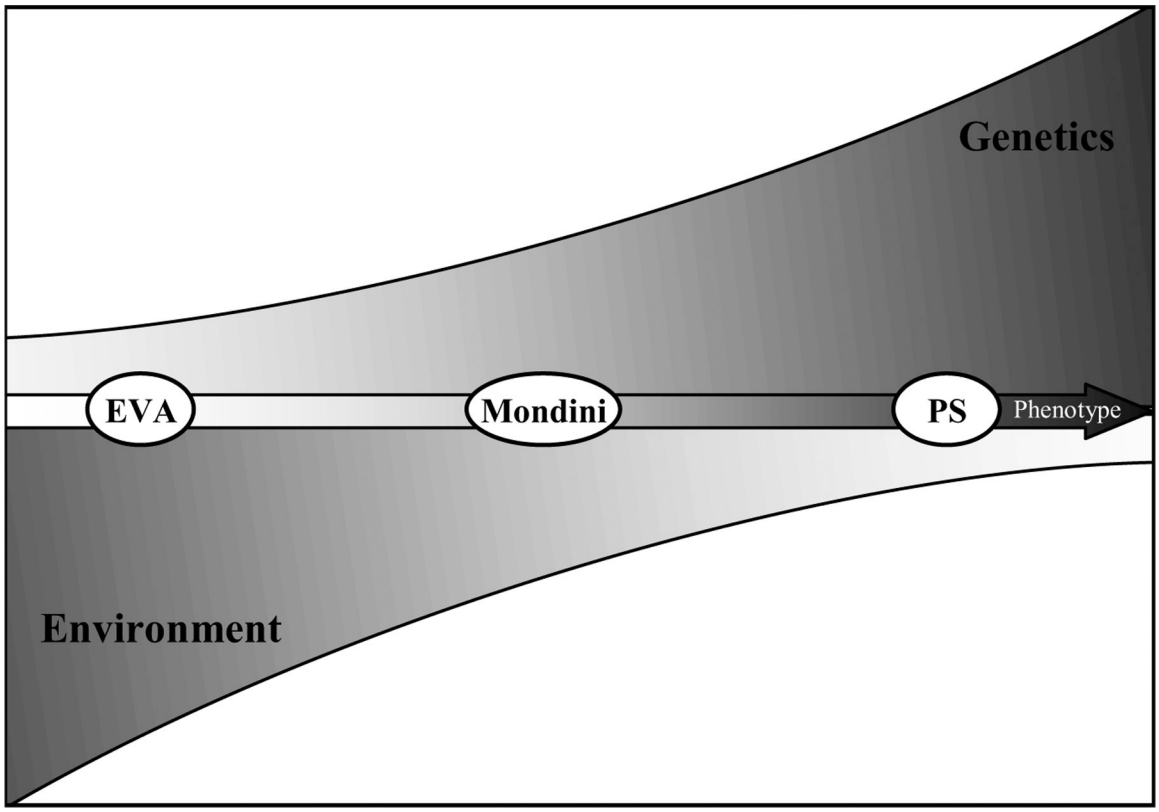

Figure 4:

Schematic illustration of the interaction of genetic and environmental factors in the etiology of EVA, Mondini and PS. Genetic heterogeneity is more pronounced in the lesser severe phenotype; EVA where the environment is believed to play a bigger role. In the other side of the spectrum, PS is more homogeneous genetically with a lesser environment influence.

Table 1:

Distribution of SLC26A4 genotypes among patients with EVA and Mondini

	EVA		Mondini	
SLC26A4 genotypes	**Bilateral**	**Unilateral**	**Bilateral**	**Unilateral**
Two mutations	28	10	27	1
One mutation	47	14	26	2
Zero mutation	178	64	54	6

Table 2:

Phenotypes and genotypes for families with EVA-Mondini.

Family	Phenotype			*SLC26A4* mutations		
	HL	**CT scan**	**Thyroid**	**2**	**1**	**0**
1	Profound	Bilateral Mondini	Goiter			*
2	Profound	Bilateral Mondini			*	
3	Profound	Bilateral Mondini		*		
4	S1-Profound S2-Unilateral profound	S1-Bilateral Mondini S2-Unilateral Mondini				* *
5	P1-Moderate S2-Unilateral Severe	P1- Bilateral EVA S2- Bilateral Mondini	P1-Abn PDT	* *		
6	Mild to profound	Bilateral EVA				*
7	Moderate	Bilateral Mondini				*
8	Profound	Bilateral Mondini		*		
9	Profound	Bilateral Mondini		*		
10	Moderate	S1-Bilateral Mondini S2-Bilateral EVA		* *		
11	Moderate	S1-Bilateral EVA S2-Unilateral EVA				* *
12	Moderate	Bilateral EVA		*		
13	Severe	Bilateral EVA		*		
14	profound	Bilateral EVA		*		

S: sibling; P: parent, Abn PDT: abnormal Perchlorate discharge test