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Identification of *SLC26A4* gene mutations in Iranian families with hereditary hearing impairment

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Abstract

Mutations in the *SLC26A4* gene at the *DFNB4* locus are responsible for Pendred syndrome and non-syndromic hereditary hearing loss (DFNB4). This study included 80 nuclear families with two or more siblings segregating presumed autosomal recessive hearing loss. All deaf persons tested negative for mutations in *GJB2* at the *DFNB1* locus and were therefore screened for autozygosity by descent (ABD) using short tandem repeat polymorphisms (STRPs) that flanked *SLC26A4*. In 12 families, homozygosity for STRPs suggested possible ABD in this genomic region. Affected individuals in 5 families had a positive perchlorate discharge test. Sequence analysis of *SLC26A4* identified 10 mutations in 8 families (T420I, 1197delT, G334V, R409H, T721M, R79X, S448L, L597S, 965insA and L445W) of which 4 are novel (T420I, G334V, 965insA and R79X). These results imply that Pendred syndrome is the most prevalent form of syndromic hereditary hearing loss in Iran.

Keywords

DFNB4; *SLC26A4* gene; Pendred; *PDS* gene

Introduction

Inherited deafness is common, occurring approximately once in every 2000 live births. The severity of this loss can vary from mild to profound [5, 9] and approaches to its investigation are varied. In many countries around the world mutations in *GJB2*, a gene at the *DFNB1* locus, are the most common cause of autosomal recessive hearing loss (ARHL) and mutation screening of this gene is recommended as an early diagnostic test.

Several studies have shown that mutations in *SLC26A4* are the second most common cause of ARHL [8]. This gene encodes a chloride-iodide transporter and is associated with both

syndromic hearing loss, Pendred Syndrome (PDS; OMIM, 605646), and non-syndromic hearing loss, DFNB4 (enlargement of the vestibular aqueduct, EVA) [6]. The encoded protein, pendrin [10], is composed of 780 amino acids and has a molecular weight of 86 kDa. It is expressed in the thyroid, inner ear and kidney.

To date, more than 90 mutations in *SLC26A4* have been described in patients with PDS and DFNB4 [2, 12]. The phenotype associated with these mutations can vary. At one extreme, there is congenital sensorineural hearing loss, thyroid dysfunction as manifested by goiter with an abnormal perchlorate discharge test, and bony abnormalities of the inner ear, typically either Mondini dysplasia or EVA, as resolved by temporal bone computed tomography. However, the presence of thyroid dysfunction is most variable, and at the other end of the phenotypic spectrum, there is congenital sensorineural hearing loss associated only with EVA.

The genetic basis for the observed phenotypic variability has not been definitively resolved. There is evidence for a functional difference between some *SLC26A4* mutations segregating with PDS versus DFNB4, however other mutations are associated with either phenotype, suggesting that other genetic or environmental factors also contribute to the final phenotype. In this study, we sought to determine the prevalence of *SLC26A4* mutations in families with autosomal recessive hearing loss (ARHL) among the Iranian deaf population.

Materials and Methods

Families

Included in this study were 80 unrelated families of consanguineous parentage segregating presumed ARHL as evidenced by the presence of at least two deaf siblings in each nuclear family. Informed consent was obtained from all persons interested in participating in this study. All affected persons had severe-to-profound deafness as determined by audiometric testing and were negative for *GJB2* and *GJB6* mutations. Evidence of thyroid dysfunction was assessed by determining thyroid size using Tc-99m scanning and thyroid function based on serum levels of TSH (normal, 0.3–3.5 mIU/L), T3 RU (normal, 25–35%), T4 RIA (normal, 4.0–13.0 ug/dl), FTI (normal, 1.1–4.5), T3RIA (normal, 80–200 ng/dl), free T3 (normal, 1.6–3.7pg/ml), free T4 (normal, 0.8–2.2 ng/dl), h Tg (normal, 2–50 ng/ml), anti-Tg (normal, 0–150 IU/ml), and perchlorate discharge (abnormal or positive result, release of more than 20% of radioactive iodide). The possibility of mutations in *SLC26A4* was evaluated by screening for autozygosity by descent (ABD) using short tandem repeat polymorphisms (STRPs) that flank *SLC26A4*. Approval for this study came from the ethical committees at the University of Social Welfare and Rehabilitation Sciences and the University of Iowa.

Mutation Screening

Each person donated approximately 10 cc of whole blood from which DNA was extracted according to standard protocols. STRPs D7S496, D7S523, D7S501 and D7S1817 were resolved in affected persons from each family. Amplification conditions were 95 °C for 5 min, followed by 30 cycles each of 94 °C for 40 s, 57 °C for 30 s and 72 °C for 40 s. PCR

products visualized using silver nitrate staining of an 8% acrylamide gel after separating amplicons for 2.5 h at 200 V. If STRP scoring was consistent with ABD, mutation screening of *SLC26A4* was completed. All 21 exons of this gene were amplified by *PCR* as described by Coyle et al (1) and bidirectionally sequenced using an automated sequencing system (*ABI* 3130XL Sequencer).

Results

As shown in Table 1, of the 80 families included in this study, presumed ABD to the genomic region that includes *SLC26A4* based on STRP analysis was present in 12. By mutation screening of *SLC26A4* we were able to identify 10 mutations (T420I, 1197delT, G334V, R409H, T721M, R79X, S448L, L597S, 965insA and L445W) in 8 of these families. In each of these 8 families, mutations were identified on both alleles. We found no mutations in *SLC26A4* in four families.

Goitrous changes of the thyroid gland were present in all 12 families and in 3, there was evidence of hypothyroidism. The perchlorate discharge test was positive in persons from 5 different families, with loss of between 23%–63% of the unincorporated iodide 2h after administration of 1 gram KCLO₄. Affected persons in each of these families also had EVA. In aggregate, in the 8 families segregating *SLC26A4* mutations, the type of goiter and degree of thyroid dysfunction varied. In only one family, with two affected members (T721M homozygotes), was the PDT markedly positive (>50%).

Discussion

After *GJB2*, mutations in *SLC26A4* are the most common cause of ARHL. They account for approximately 5% of all prelingual deafness in East Asia and 5% of recessive deafness in South Asia [7]. In this study, we identified a spectrum of mutations in *SLC26A4* in Iranian families segregating ARHL. The mutations were associated with a PDS phenotype. Although 12 of 80 families (15%) were found to have ABD consistent with linkage to the *SLC26A4* genomic region, we were able to detect mutations in only 8 families (10%). In these families, we identified 10 different mutations, 4 of which are novel (T420I, R79X, G334V and 965insA). We were unable to find mutations in 4 families, which is consistent with the finding of Yang and colleagues showing that the EVA phenotype is genetically complex [13].

We did not find any correlation between the severity of hearing loss and the type of mutation in *SLC26A4*. In our patients, EVA was the only temporal bone anomaly we observed, suggesting that EVA may be more common than Mondini dysplasia in persons with PDS. Thyroid evaluation revealed some degree of post-puberty goiter, consistent with most studies, which have shown that the majority of patients with PDS develop goitrous changes around puberty. Thyroid goiter is not synonymous with hypothyroidism, and the prevalence of hypothyroidism in this study is similar to that reported in other populations [3]. Like others, we also observed intra- and inter-familial variability in thyroid manifestations.

In aggregate, our results implicate PDS as the most prevalent form of syndromic hereditary hearing loss in Iran. It is typically difficult to differentiate from non-syndromic hearing loss in children. For that reason, we would recommend mutation screening of *SLC26A4* in persons with severe-to-profound congenital hearing loss who have EVA as resolved by temporal bone computed tomography.

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Abbreviations

HHL	hereditary hearing loss
DFNB4	autosomal recessive non-syndromic hearing loss locus 4
STRP	short tandem repeat polymorphisms
SLC26A4	solute carrier family 26, member 4
ABD	autozygosity by descent
PDS	Pendred syndrome
GJB2	Gap Junction Protein
EVA	enlarged vestibular aqueduct

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Table 1Characterization of *SLC26A4* mutations

No	Family code	Phenotype (all persons also have severe-to-profound congenital deafness)	Number of affecteds	Mutation
1	L-358	Diffuse goiter, impaired TFT ¹ , positive PDT ² , EVA ³	3	T420 I ⁴ / 1197del T
2	L-1262	Diffuse goiter, impaired TFT, positive PDT, EVA	3	Homozygous G 334 V ⁴
3	L-1650	Diffuse goiter, multinodular goiter, NL ⁵ TFT, EVA	4	Homozygous R 409 H
4	L-1280	Diffuse goiter, multinodular goiter, NL TFT, positive PDT, EVA	5	Homozygous R79X ⁴
5	L-1284	Multinodular goiter, positive PDT, impaired TFT, EVA	2	Homozygous T721M
6	L-1461	Diffuse goiter, NL TFT, positive PDT, EVA	3	Homozygous L445W
7	L-1343	Diffuse goiter, NL TFT, positive PDT, EVA	2	Homozygous S448L
8	L-527	Diffuse goiter, NL TFT, negative PDT	3	L597S/965inSA ⁴
9	L-1763	Diffuse goiter, NL TFT	3	NO MUTATION
10	L-1336	Diffuse goiter, NL TFT	3	NO MUTATION
11	L-1703	Diffuse goiter, NL TFT, negative PDT	3	NO MUTATION
12	L-342	Diffuse goiter, NL TFT, negative PDT	2	NO MUTATION

¹ TFT: Thyroid Function Test (T3, T4, TSH)² PDT: Perchlorate discharge test³ EVA: enlarged vestibular aqueduct⁴ Novel mutation⁵ NL: Normal