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Two novel SLC26A4 mutations in Iranian families with autosomal recessive hearing loss

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ABSTRACT

Objective: Due to the fact that *SLC26A4* has been suggested as the second cause of hearing loss (HL) in Iran as well as many other countries, obtaining more comprehensive information about *SLC26A4* mutations can facilitate more efficient genetic services to the patients with hereditary hearing loss. This investigation aims to detect genetic cause of two Iranian families with hearing loss.
Methods: In the present study, genetic linkage analysis via 4 short tandem repeat markers linked to *SLC26A4* was performed for two consanguineous families originating from Hormozgan and Chaharmahal va Bakhtiari provinces of Iran, co-segregating autosomal recessive hearing loss and showed no *GJB2* mutations in our preliminary investigation. For identification of mutations, DNA sequencing of *SLC26A4* including all the 21 exons, exon–intron boundaries and the promoter was carried out.
Results: The results showed linkage to this gene in both families. After sequencing, two novel *SLC26A4* mutations (c.65-66insT in exon 2 and c.2106delG in exon 19) were revealed in the two studied families.
Conclusion: Results of this study stress the necessity of considering the analysis of *SLC26A4* in molecular diagnosis of deafness especially when phenotypes such as goiter or enlarged vestibular aqueduct are present.

1. Introduction

SLC26A4 (MIM 605646) maps on 7q22-31.1 (DFNB4 locus) [1] and is probably the second most common gene accounting for hereditary hearing loss (HL), after *GJB2*. The product of *SLC26A4* is pendrin, a 86 KDa protein with 780 amino acids, and a transmembrane anion transporter of Cl^-, $HCO3^-$, OH^- and I^- expressed in cochlea, thyroid and kidney [2–6]. The protein is also expressed in respiratory and mammary epithelial cells, endometrium, testis and brain, but is unlikely to play any major role in these organs [7–9].

Mutations of *SLC26A4* are associated with recessive non-syndromic HL (DFNB4) (MIM 600791) and Pendred syndrome (PS) (MIM 274600). PS is an autosomal recessive disorder and accounts for about 10% of hereditary HL in the world [10]. The clinical symptoms of the disorder can vary within and between families [11–13] and include prelingual HL, malformations of inner ear (ranging from enlarged vestibular aqueduct (EVA) to mondini dysplasia (MD)). Notably, many patients may remain euthyroid. The type and position of *SLC26A4* mutations can influence thyroid phenotype [14–16].

So far, over 170 mutations have been identified in *SLC26A4* (http://www.healthcare.uiowa.edu/labs/pen-dredandbor/slcMutations.htm). The profile and frequency of these mutations vary among different populations. Different investigations have indicated that *SLC26A4* mutations play a more important role in causing HL in Asian and Middle Eastern patients than other populations such as Cacausians. In Korea and China, these mutations account for 6.5 and 13.73% of HL, respectively [17,18]. Up to 97.9% of Chinese HL individuals with EVA or both EVA and MD carry at least one pathogenic variant in the *SLC26A4* gene [19]. About 7.2% of prelingual or congenital recessive HL in Pakistanis and 78% of non-syndromic HL with EVA in Japan are caused by *SLC26A4* mutations [20,21].

To date, only a few studies have been performed on the role of these defects in the Iranian patients [22–25]. However, there is no sufficient information yet about spectrum and frequency of *SLC26A4* mutations in Iran.

In one investigation, performed by Kahrizi et al., in 8 out of 80 (10%) Iranian deaf families ten mutations (four novels) were found

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[22]. In another study, carried out by Tabatabaiefar et al., 4 out of 37 (10.8%) HL families were linked to DFNB4 locus [24]. Sadeghi et al. found linkage to DFNB4 locus in 3 out of 40 (7.5%) families with autosomal recessive non-syndromic congenital HL [25]. These results showed that *SLC26A4* is likely to be responsible for a considerable ratio of deafness in Iran. This is an important finding in the light of the fact that HL is an extremely heterogeneous trait [26]. In the present research, two Iranian families co-segregating autosomal recessive HL were studied by genetic linkage analysis. Subsequently, molecular genetic studies of the gene *SLC26A4* were performed.

2. Materials and methods

2.1. Sampling, clinical and physical examination

This study was approved by the Institutional Review Boards of Shahrekord University of Medical Sciences. Two consanguineous families from Hormozgan (family IR1) and Chaharmahal va Bakhtiari (family IR2) provinces of Iran, with two and six deaf patients, respectively, were included in this investigation. These families had no *GJB2* mutations in a previous preliminary study [27].

Marriage in families IR1 and IR2 had occurred between second cousins (subjects IV. 1 and IV. 2) and first cousins (subjects III. 1 and III. 2), respectively. Informed consent was taken from subjects or their parents. Based on the interviews with the adult members of the two families, informational questionnaires had been filled out and pedigrees drawn in the former study [27]. All the members of both families were sampled for further molecular studies.

2.1.1. Audiological evaluation

Hearing was evaluated using pure-tone audiometric test (PTA) for air and bone conduction at different frequencies (250–8000 Hz) in all patients.

The degree of HL was considered based on PTA average at 500, 1000, 2000 and 4000 Hz: mild (21–40 dB), moderate (41–70 dB), severe (71–95 dB) and profound (>95 dB). The severity of HL was identified by the degree of HL of the better ear.

2.1.2. Radiological examination of temporal bone

For evaluation of temporal bone status, high resolution computed tomography (CT) was carried out for all patients of the two families using a Somatom Sensation Emotion 16-Slice ConFiguration (Siemens Medical Solutions, Erlangen, Germany) (Fig. 1). EVA was detected, when the diameter at the midway between the common crus and the external aperture was 1.5 mm or more [28].

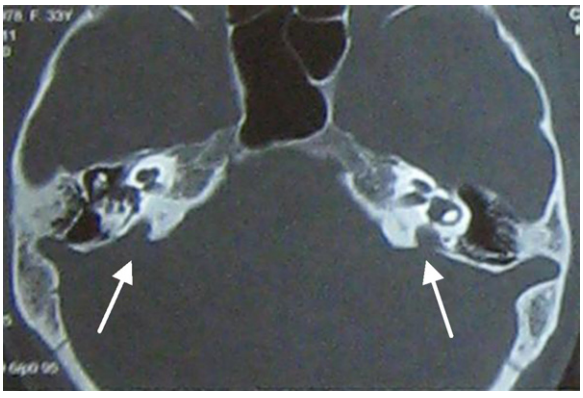

Fig. 1. Temporal bone CT scan result of patient IV. 1 (from family IR2). The arrows show enlarged vestibular aqueduct.

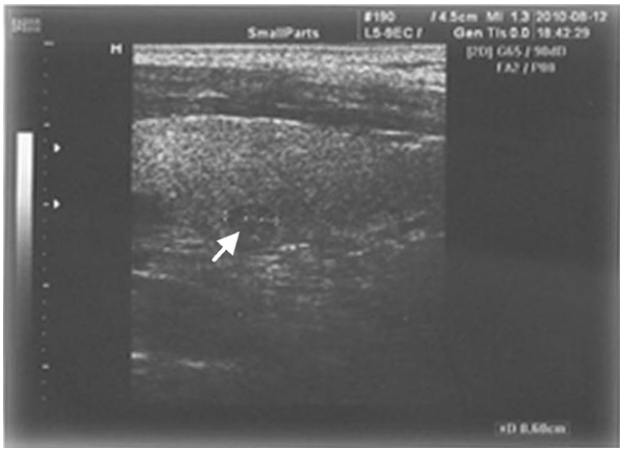

Fig. 2. Thyroid ultrasonography result of patient IV. 5 (from family IR2) with nodular goiter. The arrow shows nodule.

2.1.3. Thyroid studies

In order to investigate thyroid function in all patients of the two families, thyroid-stimulated hormone (TSH), thyroxin (T4) and triiodothyronine (T3) levels were measured by a chemiluminescent immunoassay (Berthold Technology-CSA, Germany).

As to measure the thyroid size, all patients were subject to thyroid ultrasonography using the Sonoline G50^{TM} ultrasound system (Siemens Medical Solutions, Erlangen, Germany) (Fig. 2). Thyroid ultrasonography and hormone investigation results were defined based on sex and age of patients.

2.2. Genetic analyses

2.2.1. DNA extraction

DNA was extracted from peripheral blood of all patients and other available members of the two families as well as 115 ethnically matched normal control subjects using a standard phenol chloroform method [29]. DNA quantification and quality analyses were performed by spectrophotometry (UNICO 2100, USA) and agarose gel electrophoresis using routine procedures.

2.2.2. SLink analysis and selection of DFNB4 STR markers

Four short tandem repeat (STR) markers (D7S2456, D7S2459, D7S496, and D7S2420) linked to *SLC26A4* and their primers were selected based on their physical distance at NCBI UniSTS and NCBI map viewer (http://www.ncbi.nlm.nih.gov/mapview).

SLink value for each family was calculated using FastSLink (version 2.51) option of EasyLinkage plus (version 5.05) software [30].

2.2.3. Genotyping STR markers and linkage analysis

The following amplification conditions were considered for STR markers, with some modifications for different amplicons: reactions were carried out in a 25 μl volume containing 1 μl of $MgCl_2$ (50 mM), 2.5 μl of Taq PCR buffer (10×), 0.4 μl of each of the primers (10 pM), 0.5 μl of dNTP mix (10 mM), 0.1 μl Taq DNA polymerase (5 U/μl), 2 μl DNA (50 ng), 18.1 μl ddH2O.

The touch- down PCR program for STR amplification was as follows: one cycle of 95 °C for 5 min (initial denaturation), six cycles of 95 °C for 50 s (denaturation), 58 °C for 50 s in the first cycle with 1 °C reduction per cycle (annealing), and 72 °C for 50 s (extension), 32 cycles of 95 °C for 50 s (denaturation), 53 °C for 50 s (annealing), 72 °C for 50 s (extension) and one cycle for the final extension at 72 °C for 8 min. The amplification products were run on 14% PAGE at 28 mA for 2–8 h for each marker. Silver staining was used for visualizing the bands [31].

Analysis of two-point and multi-point LOD scores were done by SuperLink (version 1.6), and SimWalk (version 2.91) options of EasyLinkage (version 5.05), respectively [30,32]. Haplotypes for STR markers were reconstructed using SimWalk and were visualized by Haplopainter software (version 029.5) [33] (Fig. 4). For calculations of LOD scores, autosomal recessive pattern of inheritance, complete penetrance and no phenocopy, equal recombination for male and female allele frequency of markers and disease allele frequency of 0.001 were assumed.

2.2.4. DNA sequencing of SLC26A4

Primers for all the 21 exons, exon–intron boundaries, and the promoter of *SLC26A4* were designed by Oligo (version 6.7.1.0 National Biosciences Inc.) (Table 1).

Amplifications of all the 21 exons and the promoter of *SLC26A4* were carried out according to the following program, with some modifications for each amplicon: one cycle of 95 °C for 5 min (initial denaturation), 36 cycles of 95 °C for 1 min (denaturation), 61 °C for 1 min (annealing), 72 °C for 1 min (extension), one cycle of 72 °C for 8 min for final extension. Each reaction was provided in a 50 μl volume containing 4 μl of MgCl2 (50 Mm), 5 μl of Taq PCR buffer (10×), 0.6 μl of each primer (10 pM), 1 μl of dNTP mix (10 mM), 0.2 μl Taq DNA pol (5 U/μl), 4 μl DNA (50 ng), 34.6 of ddH20. DNA sequencing of the PCR-amplified product was carried out bi-directionally on an ABI 3730XL automated sequencer (Applied Biosystems) (Macrogen,South Korea) using the same primers.

Table 1
Sequence of primers for 21 exons, promoter (P) and a 46 bp fragment of exon 2 (2 K) of *SLC26A4* gene.

Primer sequence (5′ → 3′)	Exon	PCR product length (bp)
F CTGGCCATTGTTCCTCTT	1	618
R TTGAGCAAGTCTCTCCCC		
F ACTCGCTTCAAGTTTGGG	2	664
R GCGAGTTTCCCAGGTAAG		
F GCACTTCAGGGTTATTATTTTC	3	461
R AAGAGAACTCTAAGGAAGGGG		
F GAAAAACAGAATGGTTGTATGG	4	496
R GAAAAAGCAGGCAAAACAC		
F GATGGGGTTTTACTATGTTGC	5	689
R CTCTCATCCTCAATTGAATCAC		
F ATTTTTGTGCTATAGGCAGG	6	459
R ATGAGGTCTCACGTCTCAAA		
F ATCACCCAGTTTTTCCTTTC	7	595
R GGGATGGATTTAACAATGC		
F ATAGACGCTGGTTGAGATTTT	8	558
R AGAAAAAAGAGCATATACGGG		
F CAGCCAGTAAGATAACACCAA	9	538
R AAAGCAAAGTGATGCAGTGT		
F TTATCGAGAGCAATGAGACC	10	606
R TCAGTTGTTATTGACCACAGC		
F TAGATGCCATTTTTGTTCAGTT	11	468
R ACACAGCTGCATAAACATCC		
F CATCTCTGCTGCGATTGT	12	556
R CCAAAGGTGTATGAATGAGC		
F AATCCAGAAGATGGAGGC	13	593
R AAATCTTAGCTCTGCCACG		
F CCAGCTGTTCATTTCAGAGT	14	378
R AAAGTTTTCATGACACTCCC		
F ACTGTGACTTGACTCCTTGC	15	321
R TTTAATTCTCATTGCCCTACAC		
F TTGTCTTTTACTGTCTTGGAGC	16	626
R TTGCACTTATTTTGTTCCTTTC		
F CACAATCATCCAGAAAACAAA	17	689
R CAGATTAAGCAACTTGCCC		
F CTGGATGTTGCCATCTCTT	18	496
R CTGTCTTTGGCCTTTTCTG		
F TAGGGTGTGCCCTGTAGTC	19	676
R TACACAAATCCCAGATCACAA		
F CAGAGGGGGTGACTTGTTA	20	628
R TAGGTATCAAATCAGGAGCAGT		
F GGGCAACAGTGAGTGAGAT	21	494
R GTGATGTAGATCAGCAGCGT		
F TGGGGAGGAGTTCTGAGT	p	715
R ATCCTCACTCATCCCGTT		
F ACAGCTGCAGCTACATGG	2k	46
R GCGAGCTCGCTGTAGACC		

2.2.5. Mutation confirmation

Pathogenicity investigation was based on the presence of homozygous variant in affected individuals and its absence in the non-affected siblings (co-segregation) of the family and absence of novel variants in the normal control persons. For investigation of c.64-65insT pathogenicity, Primers for amplification of a 46 bp fragment of exon 2, involving the location of c.65-66insT variant were designed using Oligo (version 6.7.1.0 National Biosciences Inc.) (Table 1). Exon 2 was amplified in 115 ethnically matched normal controls and all available subjects following the standard protocol mentioned above. PCR products were run on 14% PAGE at 28 mA for 3 h and silver staining was carried out to visualize the bands. Normal fragments were 1 bp smaller than fragments with the variant.

Pathogenicity of c.2106delG in exon 19 was investigated by PCR-RFLP method. The restriction enzyme Alu1 was selected for RFLP analysis of exon19, using Nebcutter (version 2.0 http://tools.neb.com/NEBcutter2). After amplification of exon 19 in 115 ethnically matched normal control and all available members of IR1 family, amplified fragments were digested and loaded on 8% PAGE at 30 mA for 2 h, followed by visualization using silver staining. After digestion normal allele had 215, 188, 177, 58, 23 and 15 bp but mutant allele had 402, 177, 58, 23 and 15 bp fragments. Therefore normal and mutant alleles were different for 402, 215 and 188 bp fragments.

3. Results

3.1. Audiological data

In family IR1, patient V. 1 had bilateral severe and V. 3 had bilateral moderate to severe HL. All patients of family IR2 had bilateral severe to profound deafness (Table 2).

3.2. Radiological information of temporal bone

EVA was determined in all affected members from two families (Table 2).

3.3. Thyroid status

All patients had normal TSH, T4, T3 hormone levels except IV. 13, who had hypothyroidism. Ultrasonography detected nodular goiter in patient V. 1, but not in patient V. 3, from family IR1 and in all of the patients from family IR2 (Table 2).

3.4. Linkage analysis results

SLink value theoretically predicts the LOD score for a family. SLink, two-point and multi-point LOD scores were 1.98, 1.49 and 2.24 for family IR1 and 3.88, 3.72 and 4.12 for family IR2, suggesting possible linkage to DFNB4. The haplotypes of the two families have been shown in Fig. 3.

3.5. SLC26A4 sequence analysis

DNA sequencing revealed two novel variants (c.65-66insT and c.2106delG) in the *SLC26A4* gene (Fig. 4). The variant c.65-66insT, in exon 2, was detected in family IR2. All patients were homozygous and individuals III. 1–III. 4 and IV. 4, IV. 10 and V.

Table 2
Characters of all patients of IR1 and IR2 families.

Family	Subject no.	Age (year)	Sex	Audiogram	CT scan	Thyroid hormone	Goiter	Variant	Homozygote or Heterozygote
IR1	V. 1	30	[a]M	Severe	[c]EVA	Normal	Nodular goiter	c.2106 delG	Homozygote
	V. 3	23	M	Moderate to severe	EVA	Normal	No goiter	c.2106 delG	Homozygote
IR2	IV. 1	32	[b]F	Severe to profound	EVA	Normal	Nodular goiter	c.65-66insT	Homozygote
	IV. 5	27	M	Severe to profound	EVA	Normal	Nodular goiter	c.65-66insT	Homozygote
	IV. 6	24	M	Severe to profound	EVA	Normal	Nodular goiter	c.65-66insT	Homozygote
	IV. 7	21	F	Severe to profound	EVA	Normal	Nodular goiter	c.65-66insT	Homozygote
	IV. 8	40	F	Severe to profound	EVA	Normal	Nodular goiter	c.65-66insT	Homozygote
	IV. 13	35	F	Severe to profound	EVA	Hypoth-yroid	Nodular goiter	c.65-66insT	Homozygote

[a] M = male.
[b] F = female.
[c] EVA = enlarged vestibular aqueduct.

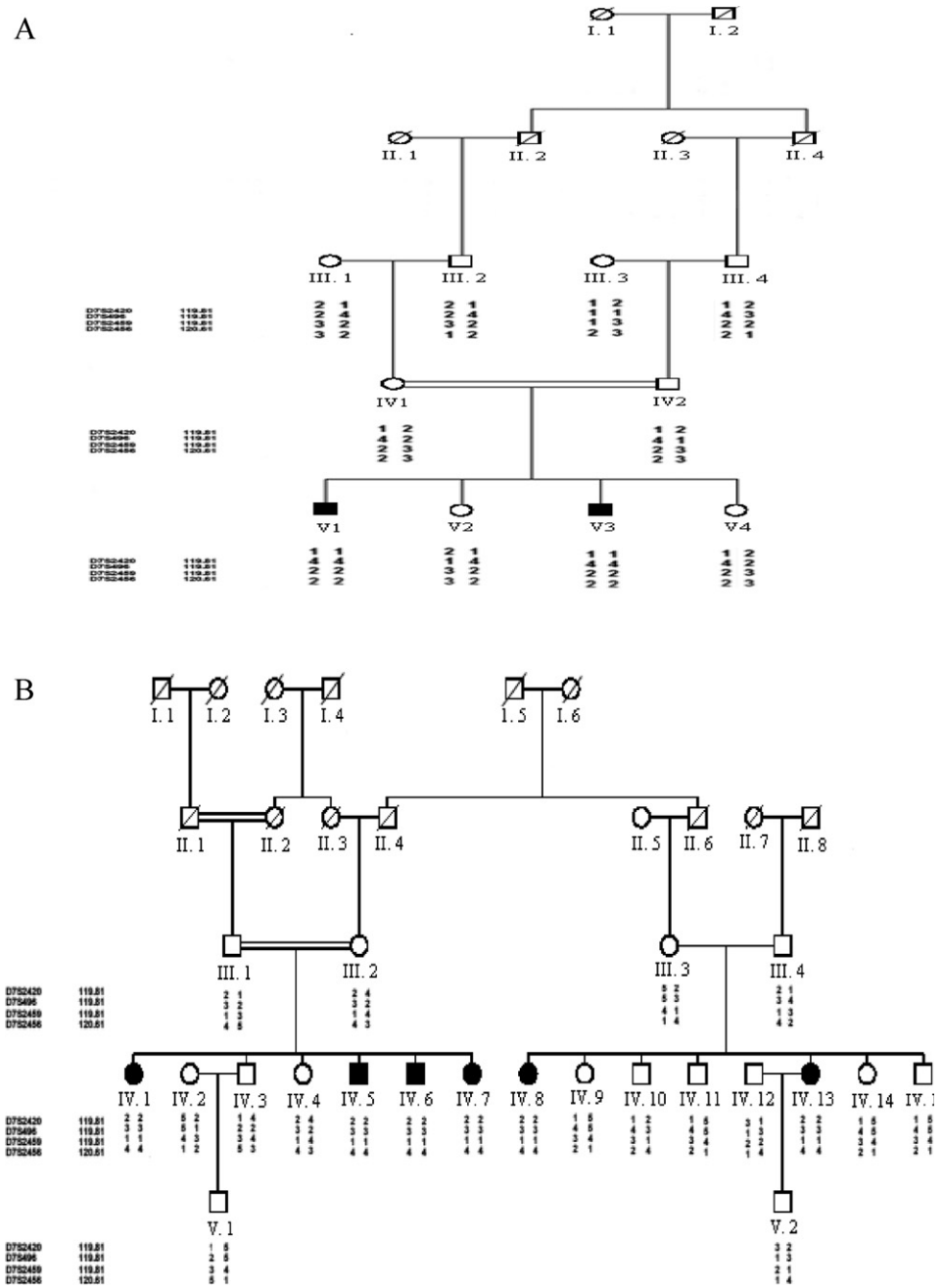

Fig. 3. Pedigrees and haplotypes of IR1 (A) and IR2 (B) families. Subjects I. 1, I. 2, II. 1–II. 4 from IR1 and I. 1–I. 6, II. 1–II. 8 from IR2, were not available for genotyping in this study. The order of markers is according to the Marshfield map. Both families show linkage to the DFNB4 locus.

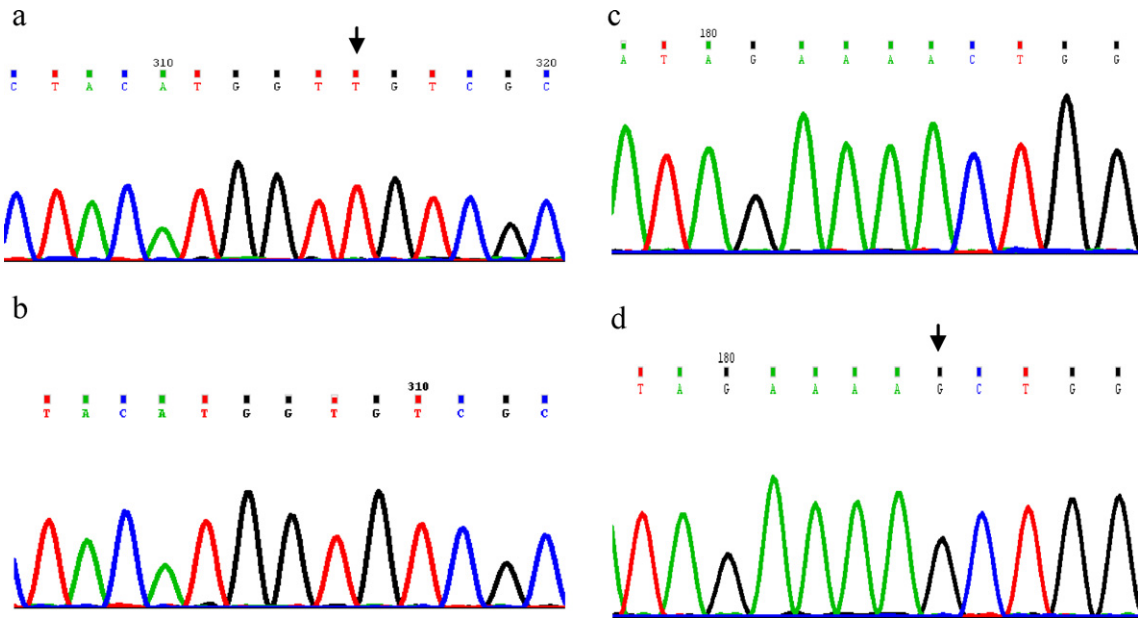

Fig. 4. Electropherograms of mutant (a) and normal (b) alleles of c.65-66insT and mutant (c) and normal (d) alleles of c.2106delG.

2 were heterozygous for the variant. All other subjects did not carry the variant. The second variant, c.2106delG in exon 19, was found in family IRI. Patients V. 1 and V. 3 were homozygous and subjects III. 1, III. 2, III. 4, IV. 1 and IV. 2 were heterozygous for this variant.

3.6. Mutation confirmation analysis

The c.65-66insT and c.2106delG variants were not detected in 115 ethnically matched control persons, without HL and co-segregated with the disease in the family. The c.65-66insT variant leads to occurrence of a frameshift from codon 23 and a premature stop at codon 86 (p.Ser 23ValfsX64). The c.2106delG variant results in a frameshift from codon 702 and premature stop at codon 720 (p.Lys702AsnfsX19). Therefore, these variants produce defective shorter proteins. These results suggest that c.65-66insT and c.2106delG are most likely to be pathogen.

4. Discussion

In the present research, we studied two families segregating severe-to-profound HL. A previous *GJB2* mutation analysis had not detected any mutations. Based on the presence of goiter, linkage analysis for DFNB4 locus was prioritized and linkage to the locus was found. Subsequent DNA sequencing of the corresponding gene, *SLC26A4*, led to the identification of two novel mutations. All patients of IR2 family were homozygous for c.65-66insT and had goiter. Although, both patients of family IR1 had homozygous c.2106delG variant, only patientV1 showed goiter. The degree of HL was also different, to some extent, between V. 1 and V. 3 patients of this family (severe in subject V. 1 and moderate to severe in V. 3).

Intrafamilial thyroid signs variability has been noted by some other investigators which makes the distinction between syndromic and non-syndromic deafness difficult [11–13]. PS and DFNB4 are characterized by HL, temporal bone anomalies. While, the development of euthyroid goiter is almost exclusive for PS [12]. However, thyroid abnormality is often not seen until the second decade of life and or even after that. Therefore, patient V. 3 from IR1 maybe show goiter at higher age.

Concordant with the human pendrin protein model, predicted by Everette et al. [1], the c.65-66insT and c.2106delG novel variants, detected in this study, change amino acids close to the N and C terminals of the protein, respectively, and could cause a complete loss of function of pendrin in c.65-66insT and in case of c.2106delG variant, would result in production of a shorter protein with defective function (Fig. 5). The hypothesis has been put forth that null or zero mutations, which abolish pendrin function completely, are associated with PS but those accompanied with reduced function can cause non-syndromic deafness [34].

Our results and other former studies emphasize that the position of *SLC26A4* mutations which affect pendrin function could, in turn, affect the function of thyroid gland [34]. Alternatively, the effect of *SLC26A4* mutations on the thyroid phenotype might be incomplete and additional parameters such as iodine uptake and modifier genes may be involved in occurrence of thyroid impairment in patients with these mutations [11–13].

Our findings suggest that c.65-66insT leads to PS and c.2106delG variant most probably leads to PS.

The c.65-66insT and c.2106delG variants lead to a mutant protein, without a big segment and C terminal portion of the protein, respectively, resulting in the production of shorter defective protein (Fig. 5) which probably cannot be processed and function correctly and does not reach the plasma membrane.

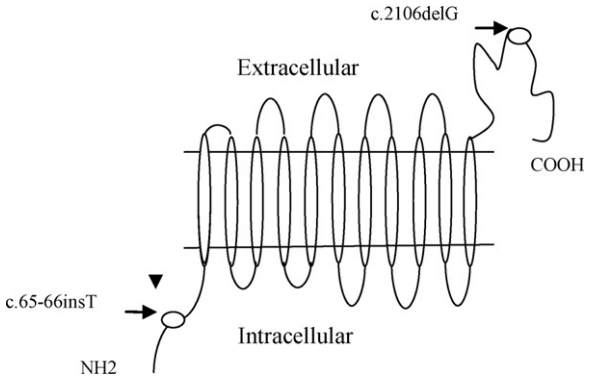

Fig. 5. Location of two novel mutations (c.65-66 insT and c.2106delG) in pendrin protein. The variants c.65-66insT in exon 2 and c.2106delG in exon 19 of *SLC26A4* lead to amino acid change close to the N terminal and C terminal of protein, respectively.

This results in abolishment of anion transporter function of pendrin. However to exactly determine the impact of c.65-66insT and c.2106delG on pendrin, more studies are warranted.

Altogether *SLC26A4* gene defects have an important role in pathogenesis of HL and are the second cause of autosomal recessive hereditary HL in various populations such as Iran [22,24,25].

Molecular study of *SLC26A4* of patients with severe to profound HL with reports of goiter in the pedigree could be important in DNA diagnosis. Since clinical and laboratory tests are non-specific, expensive and rather inaccessible, the molecular analysis has the potential to assist in the differential diagnosis of PS and DFNB4 which, in turn, could have special implications in the treatment strategy and genetic counseling in HL patients and their families.

Based on the ethnical differences in the frequencies of *SLC26A4* mutations and in order to cheaper efficient molecular diagnostic methods are designed, comprehensive data about the *SLC26A4* mutations in each population is needed.

The structure of pendrin is still challenging and has not been identified exactly. Studies of the effects of novel *SLC26A4* mutations on pendrin can provide additional beneficial information about the relation between structure-function of this protein, and other structural features can be depicted.

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References

[1] L.A. Everett, B. Glaser, J.C. Beck, J.R. Idol, A. Buchs, M. Heyman, et al., Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS), Nat. Genet. 17 (4) (1997) 411–422.
[2] D.B. Mount, M.F. Romero, The SLC26 gene family of multifunctional anion exchangers, Pflugers Arch. 447 (5) (2004) 710–721.
[3] P. Wangemann, K. Nakaya, T. Wu, R.J. Maganti, E.M. Itza, J.D. Sanneman, et al., Loss of cochlear $HCO3^-$ secretion causes deafness via endolymphatic acidification and inhibition of Ca^{2+} reabsorption in a Pendred syndrome mouse model, Am. J. Physiol. Renal Physiol. 292 (5) (2007) F1345–F1353.
[4] I.E. Royaux, K. Suzuki, A. Mori, R. Katoh, L.A. Everett, L.D. Kohn, et al., Pendrin, the protein encoded by the Pendred syndrome gene (PDS), is an apical porter of iodide in the thyroid and is regulated by thyroglobulin in FRTL-5 cells, Endocrinology 141 (2) (2000) 839–845.
[5] M.P. Gillam, A.R. Sidhaye, E.J. Lee, J. Rutishauser, C.W. Stephan, P. Kopp, Functional characterization of pendrin in a polarized cell system. Evidence for pendrin-mediated apical iodide efflux, J. Biol. Chem. 279 (13) (2004) 13004–13010.
[6] I.E. Royaux, S.M. Wall, L.P. Karniski, L.A. Everett, K. Suzuki, M.A. Knepper, et al., Pendrin, encoded by the Pendred syndrome gene, resides in the apical region of renal intercalated cells and mediates bicarbonate secretion, Proc. Natl. Acad. Sci. U.S.A. 98 (7) (2001) 4221–4226.
[7] J.A. Rillema, M.A. Hill, Prolactin regulation of the pendrin-iodide transporter in the mammary gland, Am. J. Physiol. Endocrinol. Metab. 284 (1) (2003) E25–E28.
[8] N. Pedemonte, E. Caci, E. Sondo, A. Caputo, K. Rhoden, U. Pfeffer, et al., Thiocyanate transport in resting and IL-4-stimulated human bronchial epithelial cells: role of pendrin and anion channels, J. Immunol. 178 (8) (2007) 5144–5153.
[9] L. Lacroix, C. Mian, B. Caillou, M. Talbot, S. Filetti, M. Schlumberger, et al., Na(+)/I(−) symporter and Pendred syndrome gene and protein expressions in human extra-thyroidal tissues, Eur. J. Endocrinol. 144 (2001) 297–302.
[10] A. Sanei-Moghaddam, T. Wilson, S. Kumar, R. Gray, An unfortunate case of Pendred syndrome, J. Laryngol. Otol. 125 (9) (2011) 965–967.
[11] S. Masmoudi, I. Charfedine, M. Hmani, M. Grati, A.M. Ghorbel, A. Elgaied-Boulila, et al., Pendred syndrome: phenotypic variability in two families carrying the same PDS missense mutation, Am. J. Med. Genet. 90 (1) (2000) 38–44.
[12] U. Napiontek, G. Borck, W. Muller-Forell, N. Pfarr, A. Bohnert, A. Keilmann, et al., Intrafamilial variability of the deafness and goiter phenotype in Pendred syndrome caused by a T416P mutation in the SLC26A4 gene, J. Clin. Endocrinol. Metab. 89 (11) (2004) 5347–5351.
[13] L. Fugazzola, V. Cirello, S. Dossena, S. Rodighiero, M. Muzza, P. Castorina, et al., High phenotypic intrafamilial variability in patients with Pendred syndrome and a novel duplication in the SLC26A4 gene: clinical characterization and functional studies of the mutated SLC26A4 protein, Eur. J. Endocrinol. 157 (3) (2007) 331–338.
[14] P.D. Phelps, R.A. Coffey, R.C. Trembath, L.M. Luxon, A.B. Grossman, K.E. Britton, et al., Radiological malformations of the ear in Pendred syndrome, Clin. Radiol. 53 (4) (1998) 268–273.
[15] G.N. Burrow, S.W. Spaulding, N.M. Alexander, B.F. Bower, Normal peroxidase activity in Pendred's syndrome, J. Clin. Endocrinol. Metab. 36 (3) (1973) 522–530.
[16] S.P. Pryor, A.C. Madeo, J.C. Reynolds, N.J. Sarlis, K.S. Arnos, W.E. Nance, et al., SLC26A4/PDS genotype-phenotype correlation in hearing loss with enlargement of the vestibular aqueduct (EVA): evidence that Pendred syndrome and non-syndromic EVA are distinct clinical and genetic entities, J. Med. Genet. 42 (2) (2005) 159–165.
[17] H.J. Park, S. Shaukat, X.Z. Liu, S.H. Hahn, S. Naz, M. Ghosh, et al., Origins and frequencies of SLC26A4 (PDS) mutations in east and south Asians: global implications for the epidemiology of deafness, J. Med. Genet. 40 (4) (2003) 242–248.
[18] Y. Yuan, Y. You, D. Huang, J. Cui, Y. Wang, Q. Wang, et al., Comprehensive molecular etiology analysis of nonsyndromic hearing impairment from typical areas in China, J. Transl. Med. 7 (2009) 79.
[19] Q.J. Wang, Y.L. Zhao, S.Q. Rao, Y.F. Guo, H. Yuan, L. Zong, et al., A distinct spectrum of SLC26A4 mutations in patients with enlarged vestibular aqueduct in China, Clin. Genet. 72 (3) (2007) 245–254.
[20] S. Anwar, S. Riazuddin, Z.M. Ahmed, S. Tasneem, J. Ateeq ul, S.Y. Khan, et al., SLC26A4 mutation spectrum associated with DFNB4 deafness and Pendred's syndrome in Pakistanis, J. Hum. Genet. 54 (5) (2009) 266–270.
[21] K. Tsukamoto, H. Suzuki, D. Harada, A. Namba, S. Abe, S. Usami, Distribution and frequencies of PDS (SLC26A4) mutations in Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct: a unique spectrum of mutations in Japanese, Eur. J. Hum. Genet. 11 (12) (2003) 916–922.
[22] K. Kahrizi, M. Mohseni, C. Nishimura, N. Bazazzadegan, S.M. Fischer, A. Dehghani, et al., Identification of SLC26A4 gene mutations in Iranian families with hereditary hearing impairment, Eur. J. Pediatr. 168 (6) (2009) 651–653.
[23] M.A. Tabatabaiefar, F. Alasti, N. Peeters, W. Wuyts, M.R. Nooridaloii, M.H. Chaleshtori, et al., Novel human pathological mutations. Gene symbol: SLC26A4. Disease: Pendred syndrome, Hum. Genet. 127 (2010) 468–469.
[24] M.A. Tabatabaiefar, F. Alasti, M. Montazer Zohour, L. Shariati, E. Farrokhi, D.D. Farhud, et al., Genetic linkage analysis of 15 DFNB loci in a group of Iranian families with autosomal recessive hearing loss, Iran. J. Public Health 40 (2) (2011) 34–48.
[25] A. Sadeghi, M.H. Sanati, F. Alasti, M.H. Chaleshtori, S. Mahmoudian, M. Ataei, Contribution of GJB2 mutations and Four common DFNB loci in autosomal recessive non-syndromic hearing impairment in Markazi and Qom provinces of Iran, Iran. J. Biotechnol. 7 (2) (2009) 108–111.
[26] G. Van Camp, R. Smith. Hereditary Hearing Loss Homepage: http://hereditary-hearingloss.org/, last update: May 16, 2011.
[27] M.H. Chaleshtori, D.D. Farhud, M.A. Patton, Familial and sporadic GJB2-related deafness in Iran: review of gene mutations, Iran. J. Public Health 36 (2007) 1–14.
[28] G.E. Valvassori, J.D. Clemis, The large vestibular aqueduct syndrome, Laryngoscope 88 (5) (1978) 723–728.
[29] J. Grimberg, S. Nawoschik, L. Belluscio, R. McKee, A. Turck, A. Eisenberg, A simple and efficient non-organic procedure for the isolation of genomic DNA from blood, Nucleic Acids Res. 17 (20) (1989) 8390.
[30] T.H. Lindner, K. Hoffmann, easyLINKAGE: a PERL script for easy and automated two-/multi-point linkage analyses, Bioinformatics 21 (3) (2005) 405–407.
[31] X. Wang, W. Chen, L. Zhao, P. Zhou, X. Li, Genetic polymorphism analysis of 3 short tandem repeat loci on chromosome 21 in Chinese Han population in Shaanxi Province, Nan Fang Yi Ke Da Xue Xue Bao 28 (10) (2008) 1906–1908.
[32] M. Fishelson, D. Geiger, Optimizing exact genetic linkage computations, J. Comput. Biol. 11 (2004) 263–275.
[33] H. Thiele, P. Nurnberg, HaploPainter: a tool for drawing pedigrees with complex haplotypes, Bioinformatics 21 (8) (2005) 1730–1732.
[34] D.A. Scott, R. Wang, T.M. Kreman, M. Andrews, J.M. McDonald, J.R. Bishop, et al., Functional differences of the PDS gene product are associated with phenotypic variation in patients with Pendred syndrome and non-syndromic hearing loss (DFNB4), Hum. Mol. Genet. 9 (11) (2000) 1709–1715.