nonsense mutation W77X (231 G>A) and a homozygous missense mutation C169W (507 C>T) in 2/36 (5.55%) of studied families in the coding sequence of *GJB2* and *GJB4* genes, respectively. Furthermore, DNA screenings identified other three allelic variants in *GJB2* gene in 7/72 (9.72%) of subjects [Table 2]. Out of the 7 patients, 4 for V63G (188T>G), 2 for A78T (232 G>A), and 1 for R127H (380 A>G) were heterozygote in coding region of *GJB2* gene [Table 2 and Figure 1]. Moreover, we found two additional heterozygous variants including R103C (307 C>T) and R227W (679 C>T) in the coding region of *GJB4* gene in 2/72 (2.78%) of patients [Table 2 and Figure 2]. No evidence of digenic inheritance mode was observed between *GJB2* and the *GJB4* mutations in patients who carried heterozygous mutations.

In silico analysis of new nonsynonymous variation *c.188T>G* in gap junction beta-2 and *c.679 C>T* in gap junction beta-4 genes

Among the detected variation, two variation including c.188T>G in *GJB2* and c.679 C>T in GJB4 genes have not been previously reported. To predict the putative effects of amino acid substitutions at codon positions 63 and 227 on the structure and function of Cx26 and Cx30.3 proteins, respectively, we used PolyPhen-2 online software (http://genetics.bwh.harvard.edu/pph2/index.shtml). The substitution of valine by glycine at codon 63 of Cx26 and arginine by tryptophan at codon 227 of Cx30.3 were predicted to be deleterious.(sensitivity: 0.00; specificity: 1.00).

Table 2: Gap junction beta-2 and gap junction beta-4 gene mutations found in Iranian families

Gene	Nucleotide change	Protein change	Mutation type	Number of found in participants (based on family)	
				Heterozygous	Homozypous
GJB2	188 T > G	V63G	Missense	4	-
GJB2	232 G > A	A78T	Missense	2	-
GJB2	380 A > G	R127H	Missense	1	-
GJB2	231 G > A	W77X	Nonsense	-	1
GJB4	307 C > T	R103C	Missense	1	-
GJB4	679 C > T	R227W	Missense	1	-
GJB4	507 C > T	C169W	Missense	-	1

GJB = Gap junction beta

Linkage analysis of DFNB21, DFNB24, DFNB29, and DFNB42 loci

We failed to identify linkage of the DFNB21, DFNB24, DFNB29, and DFNB42 loci among thirty-six *GJB2* negative families. If at least three STR markers (fully informative) of a DFNB locus did not show homozygosity among the affected individuals of a family, then the locus would be determined unlinked. All members of *GJB2* negative families, including parents, deaf, and healthy siblings were heterozygous for all studied STR markers.

DISCUSSION

Owing to high genetic heterogeneity in ARNSHL, designing a general efficient genetic test for diagnosis of the precise molecular basis and genetic counseling is complicated.[14] It becomes more and more complicated because the type and frequency of pathogenic mutations are notably dependent on either ethnic or geographic origins of the populations.[15] The best approaches to overcome these obstacles are the identifying common pathogenic mutations and determining significantly associated loci in ARNSHL patients for carrier screening.[16] Hence, we designed this study to screen *GJB2* and *GJB4* mutations and to evaluate the contribution of the DFNB21, DFNB24, DFNB29, and DFNB42 loci in ARNSHL families in Southern Iran.

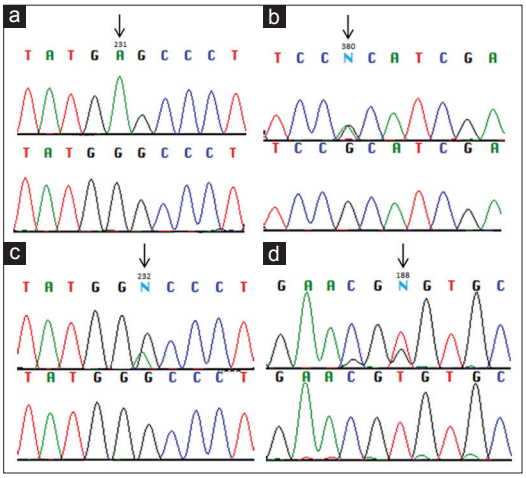

Figure 1: Sequence chromatographs of the mutated part of the gap junction beta-2 gene in proband of each family as compared to wild type. Arrows denote the mutations. (a) 231 G>A nonsense mutation. (b) 380 A>G missense mutation. (c) 232 G>A missense mutation. (d) 188 T>G missense mutation

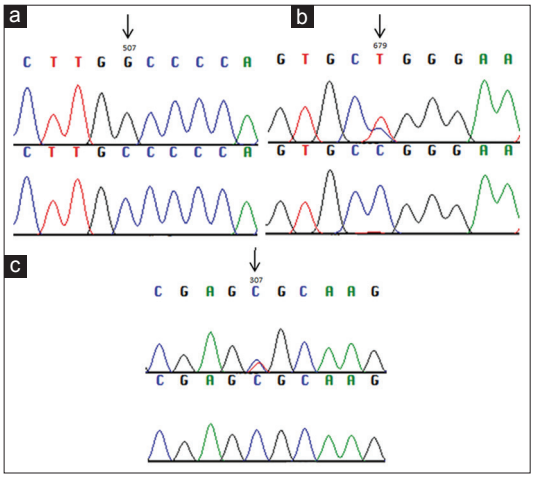

Figure 2: Sequence chromatographs of the mutated part of the gap junction beta-4 gene in proband of each family as compared to wild type. Arrows denote the mutations. (a) 507 C>T missense mutation. (b) 679 C>T missense mutation. (c) 307 C>T missense mutation

Analysis of the coding sequence of the *GJB2* gene was revealed four different allelic variants including W77X (231 G>A), V63G (188T>G), A78T (232 G>A), and R127H (380 A>G) in subjects. The pathogenic role of W77X (231 G>A), A78T (232 G>A), and R127H (380 A>G) *GJB2* mutations was previously described in various papulations[5,17] while V63G (188T>G) novel sequence variation was not reported. The W77X (231 G>A) homozygous mutation was found in one subject of studied family. This nonsense mutation occurs at the transmembrane region of Cx26, associated with moderate-to-severe HL and have been reported commonly in Indian and Pakistani families[17-19] the A78T (232 G>A) mutation which occurs in the second transmembrane domain of Cx26 was rarely detected in Indonesian and Chinese subjects.[20] The pathogenic role of R127H (380 A>G) is controversial, and several investigations have concluded that R127H most likely represents a polymorphism.[21] A novel V63G (188T>G) sequence variation was predicted as a deleterious mutation using PolyPhen software. The mutated residue lies in the first extracellular loop of Cx26 which serves as the key docking site to hemichannels at the extracellular medium.[22] According to the previous studies, it has been demonstrated that mutations in lining residue of this domain lead to disruption of voltage properties of gap junction pore.[23]

We detected a homozygous missense mutation C169W (507 C>T) in coding region of *GJB4* in one patient with severe HL phenotype. This mutation results in a substitution of cysteine to tryptophan at codon 169 of Cx30.3 protein within the second extracellular loop domain which play a fundamental role in docking of gap junction hemichannels.[24] The C169W mutation has previously been reported in Taiwanese and Iranian families.[25,26] In addition, the sequencing of *GJB4* revealed two allelic variants R103C (307 C>T) and R227W (679 C>T) variants. The association of the first variant with ARNSHL have previously been founded.[27] *In silico* analysis, demonstrated that the second variation, R227W (679 C>T), is a deleterious mutation.

We did not unveil any linkage of the DFNB21, DFNB24, DFNB29, and DFNB42 loci among *GJB2* and *GJB4* negative families. In accordance with our findings, Sadeghi *et al*. were not found evidence of linkage for DFNB21 locus in ARNSHL patients in Markazi and Qom provinces of Iran.[28] However, several investigations have reported different frequencies of DFNB21 mutations (2.7%–6.6%) in various ethnics of Iran.[29] Data from the previous studies have been indicated that mutations in *RDX* gene (DFNB24) are less common in ARNSHL cases of Middle Eastern countries such as Iran, India, and Pakistan.[9] These data are in harmony with our results. Although the mutations of *CLDN14* gene (DFNB29) were identified in Pakistani and Grecian subjects with ARNSHL, it seems that its mutations are not linked to profound deafness in Iran, Tunisia, and Turkey.[30] Up to now, more than fifteen different mutations have been detected in Pakistani, Saudi Arabian, and Czech as well as in Iranian populations.[31-33] However, we failed to find linkage of the DFNB42 locus among studied families.

CONCLUSION

In this study, we observed seven mutations in the coding region of *GJB2* and *GJB4* genes that three of them (W77X, A78T, and R127H) in *GJB2* and two mutations (R103C and C169W) in *GJB4* have been reported worldwide. We also found two novel allelic variations including V63G (188T>G) and R227W (679 C>T) in coding region of *GJB2* and *GJB4* genes, respectively. *In silico* analysis predicted that both novel variations are deleterious mutation. In contrast to several publications from Iran, we did not unveil any linkage of the studied loci among *GJB2* and *GJB4* negative families. Observed disagreement between the reports, highlights the diversity of the Iranian population and the fact that the contribution of other loci should be investigated in South of Iran.

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Conflicts of interest

There are no conflicts of interest.

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