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Genotyping Data and Novel Haplotype Diversity of STR Markers in the *SLC26A4* Gene Region in Five Ethnic Groups of the Iranian Population

Marjan Mojtabavi Naeini,[1,2] Hamzeh Mesrian Tanha,[2]
Morteza Hashemzadeh Chaleshtori,[1,*] and Sadeq Vallian[2,*]

Background and Aims: SLC26A4 gene mutations are the second currently identifiable genetic cause of autosomal recessive nonsyndromic hearing loss after *GJB2* mutations. Because of the extensive size of the *SLC26A4* gene and the variety of mutations, indirect diagnosis using linkage analysis has been suggested. Therefore, in this investigation three potential short tandem repeat (STR) markers related to this region including D7S2420, D7S496, and D7S2459 were selected for further analysis. *Methods:* The characteristics and haplotype frequency of the markers were examined for the first time in five ethnic groups of the Iranian population including Fars, Azari, Turkmen, Gilaki, and Arab using the polymerase chain reaction followed by fluorescent capillary electrophoresis. Results were analyzed by GeneMarker HID Human STR Identity, GenePop, Microsatellite tools, PowerMarker 3.25, and Arlequin 3.5 software. *Results:* Analysis of the allelic frequency revealed the presence of 11, 10, and 8 alleles for D7S2420, D7S496, and D7S2459 markers, respectively, in the Iranian population. The detailed analysis of each ethnic group was reported. Calculated polymorphism information content values were above 0.7 in the Iranian population. Pairwise linkage disequilibrium (LD) revealed a significant LD in pairing markers of D7S2420-D7S496 and in D7S496-D7S2459. Estimation of the haplotype frequency showed the presence of 20, 13, 15, 15, and 20 informative haplotypes in Fars, Azari, Turkmen, Gilaki, and Arabian ethnics, respectively. *Conclusion:* Together, the investigated markers could be suggested as powerful tools for linkage analysis of *SLC26A4* gene mutations in the Iranian population.

Background

Nonsyndromic sensorineural hearing loss (NSHL) accounts for ~70% of inherited hearing impairments (Mehl and Thomson, 1998). Autosomal recessive deafness (DFNB genes) with 75–80% frequency is the most common type of NSHL (Hone and Smith, 2003). Extreme heterogeneity and a relatively small role of each gene make it infeasible to screen mutations of all involved genes in inherited deafness (Smith and Robin, 2002). As reported, *SLC26A4* gene (OMIM *605646) mutations were the second most frequently identifiable genetic cause of autosomal recessive nonsyndromic hearing loss (ARNSHL) after *GJB2* mutations (Smith and Robin, 2002). The *SLC26A4* gene is located at 7q31 and contains 21 exons that encode a 780 amino acid protein with chloride, iodide, and bicarbonate transporting function named Pendrin (Swiss Prot accession No. O43511) (Royaux *et al.*, 2001). More than 170 mutations associated with ARNSHL have been identified within the *SLC26A4* gene, their spectrum and their frequency varied due to ethnic background (Tabatabaiefar *et al.*, 2012; Yazdanpanahi *et al.*, 2012; Yao *et al.*, 2013). Moreover, the extensive size of the *SLC26A4* gene and presence of highly linked polymorphic markers suggested that linkage analysis could be more cost effective than direct mutation detection.

Investigation of the *SLC26A4* gene region revealed the presence of different polymorphic markers including short tandem repeats (STRs). Among reported STRs in the *SLC26A4* gene region, D7S2420 (Probe accession No. Pr012582364), D7S496 (Probe accession No. Pr012475069), and D7S2459 (Probe accession No. Pr012618656) were suggested to have a high degree of heterozygosity and allelic number, which makes the markers ideal for indirect diagnosis (Gyapay *et al.*, 1994). However, to date the reported data on these STRs were restricted and no specific investigation has been performed in the Iranian population.

In view of the population-dependent nature of the polymorphic markers, the prerequisites of using STRs in linkage analysis are determination of allele and haplotype frequencies, genetic linkage, and linkage disequilibrium (LD) status

[1]Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.
[2]Genetics Division, Department of Biology, Faculty of Science, University of Isfahan, Isfahan, Iran.
*These two authors contributed equally to the article.

of the markers under study (Inturri *et al.*, 2011). Application of informative markers in linkage analysis approaches could increase the accuracy of carrier detection and prenatal diagnosis (Hildebrand *et al.*, 1992). In addition, the investigation of markers as informative haplotypes could improve the power of linkage analysis for transmission study of the affected alleles in pedigrees instead of using markers separately (Fazeli and Vallian, 2009). In this study, three STR markers of the *SLC26A4* gene region, D7S2420, D7S496, and D7S2459, were investigated for the first time in the Iranian population.

Materials and Methods

Sampling, DNA extraction, and genotyping

Blood samples were collected from 165 unrelated healthy individuals in the Iranian population. Total equal number of Fars subjects from Isfahan, Azari from Tabriz, Turkmen from Gorgan, Gilaki from Rasht, and finally Arabs from Ahvaz were used in this study. In this study, written informed consent was obtained from all involved participants or guardians. Genomic DNA was extracted by standard phenol-chloroform method. DNA concentration and purity were qualified by Nano Drop 1000 spectrophotometer (Thermo Scientific, Inc., Wilmington, DE).

DNA samples were genotyped for D7S2420, D7S496, and D7S2459 at *SLC26A4* gene region using fluorescent polymerase chain reaction (PCR) with previously described primers in the Probe Database (www.ncbi.nlm.nih.gov/probe/). Standard cycling was performed in a thermo cycler (ASTEC PC-818; ASTEC, Fukuka, Japan) with the following condition: initial denaturation at 96°C for 2 min followed by 35 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, and finally 72°C for 7 min. For fragment analysis, 5′ end of reverse primers were labeled with different fluorescent dyes (D7S2420, ROX; D7S496, HEX; and D7S2459, 6-Fam). Genotypes of STR markers were determined by fluorescent capillary electrophoresis with 16-capillary ABI 3130 Genetic Analyzer (Applied Biosystems, Carlsbad, CA). About 1.2 μL PCR products, 9 μL Hi-Di™ formamide (Applied Biosystems), and 0.3 μL Genescan™-500 LIZ™ size standards (Applied Biosystems) were diluted and loaded into capillaries with POP-4™ (Applied Biosystems) separation matrix. Finally, alleles were assigned by GeneMarker® HID Human STR Identity software.

Statistical analysis

GenePop Website (Raymond and Rousset, 1995) was used to perform the following statistical analysis, including allele frequency, observed and expected heterozygosity, and the

Table 1. Allele Frequency of D7S2420, D7S496, and D7S2459 Markers in Five Ethnic Groups of the Iranian Population

Marker	*Number*		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
D7S2420	Allele	(bp)	272	276	278	280	282	284	286	288	290	292	294
		(CA_n)	18	20	21	22	23	24	25	26	27	28	29
Iranian population	Frequency (%)		0.91	0.91	6.07	8.18	15.45	20.30	15/76	24.24	6.36	1.32	0.30
Fars			—	3.03	10.61	7.58	16.67	13.64	15.15	22.73	10.61	—	—
Azari			—	1.52	7.58	10.61	21.21	15.15	16.67	24.24	3.03	—	—
Turkmen			—	—	6.06	6.06	7.58	22.73	22.73	25.76	1.52	6.06	1.52
Gilaki			1.52	—	4.55	6.06	15.15	24.24	16.67	22.73	7.58	1.52	—
Arabian			3.03	—	1.52	10.61	16.67	25.76	7.58	25.76	9.09	—	—
Marker	*Number*		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	—
D7S496	Allele	(bp)	121	127	129	131	133	135	137	139	141	143	—
		(CA_n)	12	15	16	17	18	19	20	21	22	23	—
Iranian population	Frequency (%)		0.30	0.30	15.15	3.94	16.36	10.61	41.52	6.97	3.94	0.91	—
Fars			—	—	25.76	3.03	10.61	15.15	37.88	7.58	—	—	—
Azari			1.52	—	13.64	12.12	21.21	9.09	36.36	4.55	1.52	—	—
Turkmen			—	—	7.58	3.03	12.12	10.61	40.91	9.09	13.64	3.03	—
Gilaki			—	—	6.06	1.52	12.12	10.61	51.52	12.12	4.55	1.52	—
Arabian			—	1.52	22.73	—	25.76	7.58	40.91	1.52	—	—	—
Marker	*Number*		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	—	—	—
D7S2459	Allele	(bp)	140	142	144	146	148	150	152	154	—	—	—
		(CA_n)	16	17	18	19	20	21	22	23	—	—	—
Iranian population	Frequency (%)		0.30	3.03	27.88	3.64	30.61	23.94	10.30	0.30	—	—	—
Fars			—	1.52	43.94	1.52	25.76	21.21	6.06	—	—	—	—
Azari			1.52	1.52	19.70	4.55	27.27	30.30	13.64	1.52	—	—	—
Turkmen			—	6.06	27.27	6.06	34.85	24.24	1.52	—	—	—	—
Gilaki			—	3.03	27.27	4.55	30.30	16.67	18.18	—	—	—	—
Arabian			—	3.03	21.21	1.52	34.85	27.27	12.12	—	—	—	—

exact and chi-square test for Hardy–Weinberg equilibrium (HWE). Polymorphism information content (PIC) was estimated by using the Microsatellite Toolkit (Park, 2001). LD between markers was calculated with PowerMarker 3.25 software (Liu and Muse, 2005), and frequency estimation was performed by Arlequin 3.5 software (Excoffier *et al.*, 2005).

Results

The allele frequency of genetic markers including D7S2420, D7S496, and D7S2459 in *SLC26A4* gene region was estimated in a group of healthy individuals in five ethnic groups of the Iranian population as listed in Table 1. Genotyping analysis of D7S2420 revealed the presence of 11 alleles in the Iranian population (Fig. 1). The genotyping data for different ethnic groups were shown Table 1. As illustrated in Figure 1, PCR products of alleles ranging from 272 to 290 bp correspond to alleles with 18–27 CA core repeats. Moreover, D7S496 marker in the Iranian population showed 10 alleles with size of 121–143 bp, representing 12–23 CA core repeats (Fig. 1). Finally, genotyping analysis of D7S2459 marker indicated the presence of eight alleles in the studied population. For this marker, the alleles ranged from 140 to 154 bp correspond to 16–23 CA core repeat alleles (Fig. 1).

The observed and expected frequencies of heterozygosity, HWE *p*-values, and PIC values for the analyzed markers were calculated. As shown in Table 2, the observed heterozygosity of the three markers differed in five ethnic groups. The degree of heterozygosity for D7S2420, D7S496, and D7S2459 was 82.4%, 74.5%, and 70.3%, respectively. The calculated HWE *p*-values for all the examined markers were above 0.05, except for Fars ethnic of D7S2459 marker which was not considered in HWE assumption-based analysis. The informativeness of the markers was examined by analysis of PIC value. The PIC values for D7S2420 and D7S2459 were above 0.7 in all the Iranian ethnic groups, indicating that the markers could be considered as highly informative. However, D7S496 was found highly informative (above 0.7) only in Fars, Azari, and Turkmen, but only moderately informative in Gilaki and Arabian ethnic groups (Table 2).

LD analysis for three linked markers was performed between two adjacent pairs. As shown in Table 3, based on different genetic structures, *p*-value of exact test and chi-square *p*-value were different in different ethnic groups. However, the Iranian population (five ethnic groups) showed significant LD *p*-value of exact test < 0.000001 for D7S2420-D7S496 markers pair. Moreover, the relative value for D7S496-D7S2459 pair was 0.0014.

Ultimately, haplotype frequency was calculated in three groups. The informative haplotypes (frequency above 5%) were represented in Table 4. Although the analysis revealed different informative haplotypes in different ethnics, 10 informative haplotypes concurrently existed in 5 ethnic groups of the Iranian population.

Discussion

Mutations in *GJB2* and *SLC26A4* genes have been reported as the most common causes of ARNSHL worldwide (Smith and Robin, 2002). Unlike *GJB2*, *SLC26A4* is a large gene with a variety of known ARNSHL pathogenic mutations without

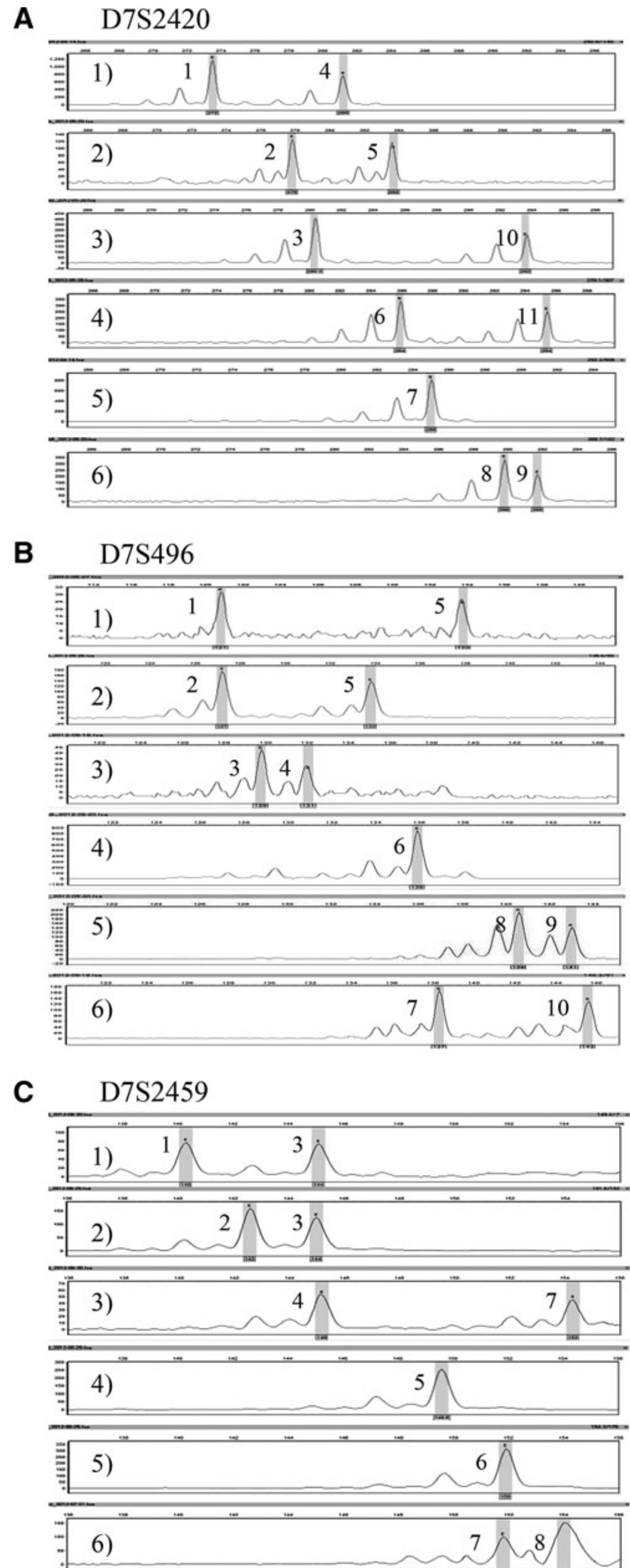

FIG. 1. Genotyping of **(A)** D7S2420, **(B)** D7S496, and **(C)** D7S2459 markers in the five ethnic groups of the Iranian population. Allele numbers are described in Table 1.

Table 2. Estimation of Observed Heterozygosity, Expected Heterozygosity, HWE *p*-Value, and PIC Value for D7S2420, D7S496, and D7S2459 Markers in the Iranian Population

Marker	*Population*	*Observed heterozygosity (%)*	*Expected heterozygosity (%)*	*HWE* p-*value*	*PIC value*
D7S2420	Iranian (5 ethnics)	82.4	83.9	0.3130	0.816
	Fars	87.9	86.3	0.7163	0.832
	Azari	78.8	84.0	0.5893	0.804
	Turkmen	84.8	82.6	0.5589	0.788
	Gilaki	81.8	84.0	0.4339	0.804
	Arabian	78.8	82.6	0.7710	0.788
D7S496	Iranian (5 ethnics)	74.5	76.1	0.3325	0.731
	Fars	78.8	76.1	0.0504	0.712
	Azari	72.7	71.9	0.2822	0.750
	Turkmen	81.8	78.4	0.8166	0.749
	Gilaki	69.7	69.8	0.6957	0.661
	Arabian	69.7	71.9	0.1230	0.659
D7S2459	Iranian (5 ethnics)	70.3	76.1	0.0917	0.718
	Fars	51.5	70.2	0.0003	—
	Azari	84.8	78.6	0.6099	0.738
	Turkmen	63.6	74.9	0.0831	0.700
	Gilaki	78.8	78.2	0.4187	0.733
	Arabian	72.7	75.7	0.7041	0.700

HWE, Hardy–Weinberg equilibrium; PIC, polymorphism information content.

any prevailing frequency (Tabatabaiefar *et al.*, 2011). Moreover, *SLC26A4* mutations of ARNSHL differ among populations around the world (Tabatabaiefar *et al.*, 2012). Consequently, it is preferable to use linkage analysis to facilitate molecular diagnosis of *SLC26A4*-based ARNSHL. Linkage analysis requires informative polymorphic markers and haplotypes that are specific and allocated within populations (Terwilliger, 1995). In this, three STR markers within *SLC26A4* gene region were chosen (D7S2420, D7S496 extragenic, and D7S2459 intragenic) and their characteristics were precisely analyzed within five ethnic groups of the Iranian population.

Investigation of the D7S2420 marker revealed the existence of at least eight alleles in each ethnic group (Table 1). Among the alleles, the allele number 8 was the most frequent (above 22%) in four ethnicities (Fars, Azari, Turkmen, and Arabian), whereas the allele 6 showed highest frequency (25.73%) in the Gilaki. This allele showed the same frequency as allele 8 in the Arabian group. Therefore, allele 8 could be considered as the most frequent allele in the Iranian population (24.24%). Based on the reports from the Genethon Center (www.genethon.fr/) and the Mammalian Genotyping Service (http://research.marshfieldclinic.org/genetics/home/), the polymorphic range for D7S2420 marker was 272–292 bp, which equals to alleles 1–10 in this study (Table 1). Interestingly, the allele with 294 bp (allele 11) is a new allele for the D7S2420, which was identified in Turkmen in the Iranian population. HWE analysis demonstrated no deviation at D7S2420 loci in any of the ethnics, and the *p*-values were above the expected threshold ($p > 0.05$). The PIC of all ethnics was between 0.788 and 0.832, which could identify this marker as a highly informative polymorphism in the *SLC26A4* gene region in the Iranian population.

Table 3. Results of Exact Test and Chi-Square Test *p*-Values of Linkage Disequilibrium

Locus pairs	*Population*	p-*Value*	χ^2 p-*Value*
D7S2420-D7S496	Iranian (5 ethnics)	**0.0000**	**0.0000**
	Fars	**0.0004**	0.0270
	Azari	0.0570	**0.0018**
	Turkmen	0.3150	**0.0000**
	Gilaki	0.1630	0.0435
	Arabian	**0.0000**	**0.0002**
D7S496-D7S2459	Iranian (5 ethnics)	**0.0014**	**0.0087**
	Fars	—	—
	Azari	0.0550	0.0230
	Turkmen	0.4590	0.0328
	Gilaki	**0.0040**	**0.0000**
	Arabian	0.5900	0.0104

Bold values are significant values.

D7S496 polymorphic marker was reported with allele size ranging 129–141 bp in UniSTS Probe Data Bank (www.ncbi.nlm.nih.gov/probe/). However, three additional alleles (alleles 1, 2, and 10, correspond to 121, 127, and 143 bp) were found in the present investigation that have not been reported previously. Allele 1 and 2 and 10 were identified in Azari, Arabian, and the two ethnics of Turkmen and Gilaki, respectively. However, allele 7 with frequencies between 36.36% and 51.51% was the most frequent allele identified in the five ethnic groups of the Iranian population. HWE distribution analysis revealed no deviation among any of the ethnic groups, which verifies further analysis on this marker. In general, the PIC values demonstrated high informativeness of D7S496 marker for linkage analysis in the Iranian population, especially, in detailed prospect for Fars, Azari, and Turkmen individuals. Moreover, D7S496 marker could be considered as a moderate informative marker for the Gilaki and Arabian ethnics based on their values (0.659, 0.661).

Data analysis of the D7S2459 marker clarified the presence of the 154 bp allele among the Azari (allele 8) in addition to

Table 4. Informative Haplotypes and Frequencies of D7S2420, D7S496, and D7S2459 Markers in the Iranian Population

Pairs of markers	*Population*	*Informative haplotype*	*Frequency*
D7S2420-D7S496-D7S2459	Iranian (5 ethnics)	7/7/5	0.05031
	Fars	8/7/3	0.07806
		8/3/3	0.06256
		9/7/3	0.05718
	Azari	—	—
	Turkmen	7/7/5	0.10491
		6/7/3	0.06060
		6/9/6	0.05945
	Gilaki	6/7/5	0.10937
		8/7/5	0.09091
	Arabian	6/7/5	0.12848
		6/3/6	0.07185
		7/7/5	0.07184
		4/5/3	0.05882
		8/7/6	0.05547
D7S2420-D7S496	Iranian (5 ethnics)	6/7	0.11477
		7/7	0.09188
		8/7	0.07980
		8/3	0.06705
		5/7	0.06456
	Fars	8/3	0.10586
		6/7	0.09375
		5/7	0.08927
		9/7	0.07147
		8/7	0.06442
		8/6	0.06410
		3/5	0.06250
		7/3	0.05936
		7/4	0.05642
	Azari	7/7	0.13636
		8/3	0.09091
		5/7	0.08739
		6/7	0.07077
		4/5	0.06061
		6/6	0.05042
	Turkmen	7/7	0.12445
		6/9	0.10149
		6/7	0.09467
		8/7	0.07856
		5/7	0.07576
		7/6	0.06875
	Gilaki	6/7	0.17961
		8/7	0.14875
		7/7	0.07576
		4/5	0.06061
		5/7	0.05786
		5/6	0.05785
	Arabian	6/3	0.15917
		8/7	0.13235
		8/5	0.11764
		7/7	0.08564
		5/5	0.07871
		6/7	0.07612
		4/5	0.05365
		5/7	0.05364
D7S496-D7S2459	Iranian (5 ethnics)	7/5	0.15914
		7/6	0.11330
		7/3	0.09794
		3/5	0.05211
	Fars	7/3	0.20072
		3/5	0.10982
		7/6	0.10938
		3/3	0.10894
		7/5	0.06490
		6/6	0.06250
		6/3	0.06250
		5/5	0.05966
	Azari	7/5	0.13767
		7/6	0.11790
		5/6	0.09295
		3/6	0.07460
		5/3	0.06900
		4/3	0.05820
		7/7	0.05051
	Turkmen	7/6	0.14031
		7/5	0.13820
		7/3	0.11543
		6/5	0.07274
		8/3	0.05863
		9/6	0.05367
	Gilaki	7/3	0.17587
		7/5	0.12351
		7/6	0.11466
		7/7	0.08596
		6/3	0.07576
		3/5	0.07576
		8/7	0.05041
	Arabian	7/5	0.21747
		5/3	0.13329
		7/6	0.09757
		3/6	0.09688
		7/3	0.07883
		3/7	0.07576
		3/5	0.05463

previously reported alleles in UniSTS Probe Data Bank. Unlike the D7S2420 and D7S496 markers, the most frequent allele for D7S2459 (allele 5) differed in different ethnic groups. Allele 3 in Fars, 6 in Azari, 5 in Gilaki, Turkmen, and Arabian were the most frequent among the alleles identified. Together, allele 5 with 30.61% frequency was the most prevalent in the Iranian population. HWE *p*-values for four ethnics except Fars (five ethnics) were above 0.05. The Fars ethnic group showed deviation from HWE ($p = 0.0003$), which could be explained by possible laboratory/genotyping errors or population stratification. Therefore, in view of the deviation from HWE, further analysis of the Fars ethnic is necessarily prevented (for instance, PIC test and pairwise LD test). Furthermore, based on the results form PIC tests, the D7S2459 marker (PIC values >0.7) could be introduced as a highly informative polymorphism among the studied ethnic groups of the Iranian population.

A pairwise LD test on the adjacent pair of the studied markers was performed. The data indicated that D7S2420-D7S496 had noteworthy values ($p < 0.00001$) demonstrating significant LD in the studied population and among Fars and Arabian individuals in a detailed deliberation. On the other hand, LD analysis corroborated existence of LD for the

D7S496-D7S2459 pair ($p = 0.0014$) in the Iranian population. Moreover, LD test analysis of each ethnic group showed the highest LD for the Gilaki (p-value = 0.004, chi-square p-value < 0.00001) among the four studied ethnic groups.

Our data on the analysis of haplotype frequency indicated the presence of 20, 13, 15, 15, and 20 informative haplotypes in Fars, Azari, Turkmen, Gilaki, and Arabian ethnic groups, respectively (Table 4). However, of the informative markers, 10 markers could be considered as informative in the Iranian population, which could be suggested for application in molecular screening of the disease causing *SLC26A4*-related mutations.

Conclusion

This study for the first time provided information on D7S2420, D7S496, and D7S2459 polymorphic markers located in the *SLC26A4* gene region in the Iranian population. The estimated informative haplotypes would therefore greatly assist optimization of molecular tests for *SLC26A4* in ARNSHL mutation detection in linkage based strategies in the Iranian population.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Sadeq Vallian, PhD
Genetics Division
Department of Biology
Faculty of Science
University of Isfahan
Isfahan 88716
Iran

E-mail: svallian@sci.ui.ac.ir

Morteza Hashemzadeh Chaleshtori, PhD
Cellular and Molecular Research Center
Shahrekord University of Medical Sciences
Shahrekord 03813330709
Iran

E-mail: mchalesh@skums.ac.ir