Study And Molecular Investigation Of Transmembrane Channel-Like1 Gene Related Polymorphic Markers In Iranian Population

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Abstract: Transmembrane channel-like (TMC) gene has been already reported to cause nonsyndromic autosomal dominant and recessive hearing loss. Finding appropriate genetic Markers for mutation screening of the gene is crucial. The genetic information and population data for these STRs may be used not only in quantitative fluorescence-polymerase chain reaction assays but also in forensic studies and other genetic tests. In this study, the identity and characteristics of three CA short tandem repeat (STR) markers, including D9S1876, D9S1837 and D9S1799, related to this gene region were examined for further analysis in the Iranian population. **Methods**: The loci were genotyped by fluorescent capillary electrophoresis DNA sequencing. **Results**: Pair-wise linkage disequilibrium (LD) showed a considerable LD in paring markers Of D9S1876-D9S1837 and D9S1837-D9S1799. Based on haplotype analysis, eleven Informative haplotypes within markers with more than 5% frequency were observed in Iranian population. **Conclusion:** The introduced markers could be suggested as informative and reliable tools in Running linkage analysis of TMC1 gene mutations in the Iranian population.

Index Terms: Short tandem Repeat; Transmembrane channel-like 1, Haplotype.

1 INTRODUCTION

Hearing loss is the most common congenital sensory impairment [1], affecting 1 in 1000 Infants worldwide [2]. Approximately 70% of the infants with inherited hearing loss are nonsyndromic [3], out of whom about 80% are autosomal recessive [4]. Because of tremendous genetic heterogeneity and relatively small role of each gene, it is unlikely to screen mutations of all involved genes that affect the process of inherited deafness [5]. Transmembrane channel-like1 (TMC1) gene mutations has been already reported as one of the most common causes of autosomal recessive non-syndromic hearing loss (ARNSHL) in North Africa, the Middle East, and India [6-9]. TMC1 gene should be considered in routine diagnosis if GJB2 is negative [10]. The TMC1 gene is located on the long (q) arm of chromosome 9 at position 21.13 and contains twenty-four exons and encodes a 760 amino acids long 87.8 kDa multipass transmembrane protein [11]. TMC1 is the member of a family of genes encoding proteins of unknown function that are predicted to contain multiple transmembrane domains. TMC1 is predicted to be involved in the functional maturation of cochlear hair cells. Cochlear neurosensory hair cells of Tmc1 mutant fail to mature into fully functional sensory receptors and exhibit structural degeneration that could be result of the maturational defect. The precise function of the TMC1 protein in the inner ear is unknown, although its expression is localized to outer hair cells of the inner ear [11, 12].

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Mutations in this gene at the DFNA36 and DFNB7/11 loci have previously been reported to Cause nonsyndromic autosomal dominant and recessive hearing loss, respectively [11]. Because of large size of the TMC1 gene, the large number of identified mutations in this gene and presence of highly linked polymorphic markers, linkage analysis could be more efficient than direct mutation detection. Investigations of TMC1 gene region demonstrated the presence of various polymorphic Markers including short tandem repeats (STRs). STRs have gained importance mainly Because of high degree of polymorphism in human populations STRs, known as microsatellites, which are abundant in the eukaryotic genome. Because of their high level of polymorphism and low mutation rate, STRs are widely used as genetic markers in mapping studies, disease diagnosis, and human identity testing. [13, 14,]. Each STR acts as a marker for a particular chromosome, and thus has recently been utilized in quantitative fluorescence-polymerase chain reaction (QF-PCR) assays for the prenatal detection of common aneuploidies. STR markers vary among population groups. Therefore, STR markers must be evaluated for their polymorphisms and heterozygosity before using in QF-PCR assays for a particular population. In the present study three different STR markers including D9S1876, D9S1837 and D9S1799 were investigated. These markers have high degree of heterozygosity and allelic number, which makes them Appropriate for indirect diagnosis. Due to the fact that markers usually show a population-based, haplotype-dependent frequency, determination of allele and haplotype frequencies, genetic linkage and linkage Disequilibrium (LD) status of the markers under study have been already suggested to use STRs in linkage analysis [15]. Use of informative markers in linkage analysis could increase The precision of carrier detection and prenatal diagnosis. Moreover, the investigation of markers as informative haplotypes could improve the strength of linkage analysis for transmission of the affected alleles from parents to children instead of using markers separately [16, 17]. In this investigation, the identity and characteristics of three CA STR markers including D9S1876, D9S1837 and D9S1799 at the TMC1 region were examined for the first time in the Iranian population.

2 MATERIAL AND METHODS

2.1 Sample preparation and DNA extraction

Blood samples were collected from 165 unrelated healthy donors from the Iranian population. The total genomic DNA was extracted from the leukocytes using standard phenol-chloroform Method [18].

2.2 Genotyping and PCR amplification

The DNA samples were genotyped for D9S1876, D9S1837 and D9S1799 STR loci at TMC1 Gene region using fluorescent PCR technique with primers described in UniSTS data of Probe database (http://www.ncbi.nlm.nih.gov/probe/). Standard cycling was carried out in a Thermo cycler (ASTEC PC-818; ASTEC, Fukuoka, Japan) as following condition: initial denaturation at 96° C for 2 min followed by 35 cycles of 94° C for 30", 56° C for 30", 72° C for 30", and with a final extension at 72 °C for 7 min. For fragment analysis, 5' end of reverse Primers were labeled with different fluorescent dyes (D9S1876, HEX; D9S1837, 6-Fam; D9S1799, 6-FAM)

2.3 Sample electrophoresis and data analysis

The analysis of STR markers of each amplification product was performed by fluorescent capillary electrophoresis with 16-capillary ABI 3130 Genetic Analyzer (Applied Biosystems, California, USA). 1.2 µl amplification products, 9 µl Hi-DiTM formamide (Applied Biosystems, USA) and 0.3 µl GenescanTM-500 LIZTM size standards (Applied Biosystems, USA) were diluted and loading was carried out in capillaries with POP- 4TM (Applied Biosystems, California, USA) separation matrix. Finally, alleles were determined by Gene Marker® HID Human STR Identity software.

2.4 Statistical Analysis

For data analysis, GENPOP website [19] was used to assess Hardy-Weinberg equilibrium (HWE) by Fisher's exact test, Allele frequencies, expected heterozygosity, polymorphism information content, and probability of paternity exclusion were calculated and observed. Polymorphism information content (PIC) estimation was performed by Microsatellite toolkit [20]. POWERMARKER 3.25 software was used to calculate LD between markers [21], and Haplotype frequency was estimated by ARLEQUIN 3.5 software [22].

3 Results and Discussion

Allele frequencies of the three previously mentioned genetic markers including D9S1876, D9S1837 and D9S1799 in TMC1 gene region were investigated in a group of healthy Individuals from the Iranian population. In Table 1, the characteristics of the examined STR Loci were illustrated. As illustrated in Figure 1, in a sample of 165 unrelated Iranian Individuals, genotyping of the loci revealed 9, 11 and 15 different alleles for D9S1876, D9S1837 and D9S1799 loci, respectively. Also, for these markers, the alleles ranged from 132-154 bp corresponding to 12-23 CA core repeat alleles for D9S1876, 221-251 bp Corresponding to 8-23 CA core repeat alleles for D9S1837 and 137-177 bp corresponding to 12-32 CA core repeat alleles for D9S1799 (Figure 1). Moreover, the expected (He) and observed (Ho) heterozygosity, HWE p-values and PIC Values for the analyzed markers were calculated. As presented in Table 2, the heterozygosity Of three STR loci, D9S1876, D9S1837 and D9S1799, was 77%, 78.8% and 89.7%, Respectively. The calculated HWE p-values were above 0.05 for the analyzed loci in this population (P>0.05). The informativeness of the markers was examined by analysis of PIC value. The PIC values for the STR loci are shown in Table 2. Three STR loci were above 0.7 in this Population, indicating high informativeness of the markers. LD analysis for three linked markers was carried out between two adjacent pairs using two Parameters Fisher's exact test p-value and chi-square p-value. As shown in Table 3, the Iranian population in this study indicated significant LD p-value of Fisher's exact test <0.000001 for D89S1876-D9S1837 markers pair. Moreover, the relative value for D9S1837- D9S1799 pair was 0.0001. Finally, based on haplotype analysis, eleven informative haplotypes within markers with More than 5% frequency were observed in Iranian population. However, the combination of D9S1876-D9S1837 in 148bp-239bp with 20.17% frequency was the most frequent haplotype.

4 Discussion

In this study, three STR markers at the TMC1 gene region were chosen (intragenic D9S1876, D9S1837, and extragenic D9S1799) and their identity and characteristics were precisely examined in an Iranian population. In the present study, investigation of D9S1876 marker

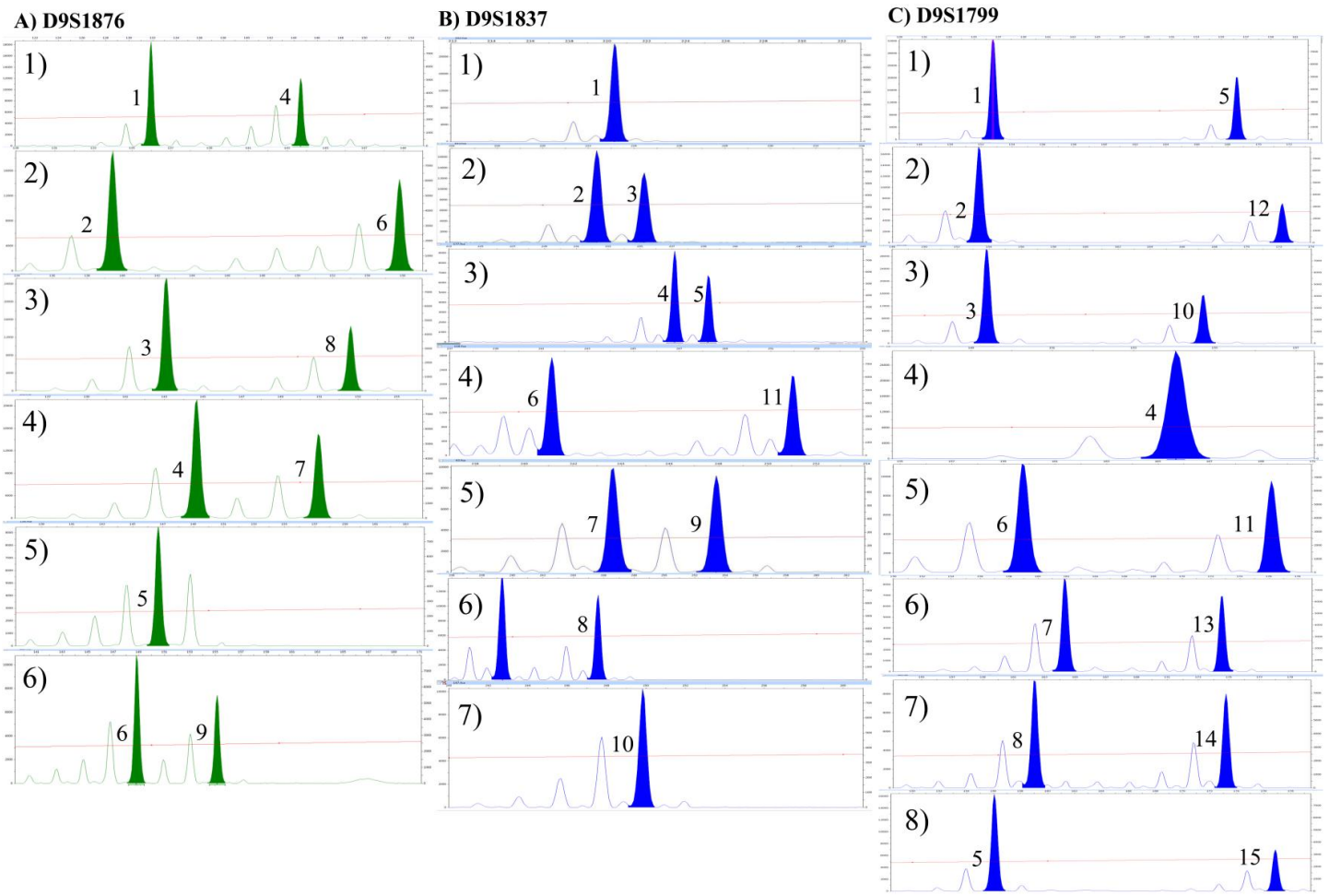

Fig 1: *Genotyping of D9S1876, D9S1837 and D9S179 markers in the Iranian population. Allele numbers are coincidence with Table 1.*

Table 1: *Allele frequency of D9S1876, D9S1837 and D9S1799 markers in Iranian population.*
**Represents the core tow-nucleotide repeat*

D9S1876			D9S1837			D9S1799		
Allele*	Frq(%)	Length(bp)	Allele*	Frq(%)	Length(bp)	Allele*	Frq(%)	Length(bp)
12	6.36	(132)	8	5.76	(221)	12	4.85	(137)
13	6.97	(134)	9	13.33	(223)	19	1.52	(151)
17	19.39	(142)	10	11.21	(225)	20	1.52	(153)
18	11.82	(144)	16	4.85	(237)	21	1.82	(155)
19	11.82	(146)	17	36.36	(239)	22	3.64	(157)
20	34.85	(148)	18	17.58	(241)	23	10.91	(159)
21	7.27	(150)	19	2.42	(243)	24	10.91	(161)
22	0.91	(152)	20	4.24	(245)	25	13.64	(163)
23	0.61	(154)	21	1.21	(247)	26	8.79	(165)
			22	1.52	(249)	27	7.88	(167)
			23	1.52	(251)	28	7.88	(169)
						29	8.79	(171)
						30	6.06	(173)
						31	6.67	(175)
						32	5.15	(177)

Table 2: *Estimation of observed heterozygosity, expected heterozygosity, HWE p-value and PIC value for D9S1876, D9S1837 and D9S1799 markers in the Iranian population.*

Marker	Observed Heterozygosity (%)	Expected Heterozygosity (%)	HWE *P*-Value	PIC Value
D9S1876	77.0	80.1	0.5191	77.5
D9S1837	78.8	80.0	0.1973	77.6
D9S1799	89.7	91.7	0.2077	90.8

Table3: *Results of exact test and chi-square test p-values of linkage disequilibrium.*

Locus pairs	*P* value	χ^2 *p* value
D9S1876- D9S1837	0.3893	0.0000
D9S1837- D9S1799	0.3634	0.0001

Table4: *Informative haplotypes and frequencies of D9S1876, D9S1837 and D9S1799 markers in the Iranian population*

Pairs of Markers	Informative Haplotype	Frequency
D9S1876-	148/239/163	0.067822
D9S1837-	142/241/173	0.050641
D9S1799	148/239/159	0.050860
	148/239	0.201742
	142/241	0.064244
D9S1876-D9S137	142/239	0.055290
	146/241	0.053566
	144/223	0.527880
D9S1837-D9S1799	239/163	0.070728
	239/159	0.066864
	241/179	0.064669

Indicated the presence of at least nine Alleles in the Iranian population (Table 1). Among the alleles, the allele 6 showed the highest Frequency (34.85%). Therefore, allele 6 could be considered as the most frequent allele in the Iranian population. According to the UniSTS reports of Probe database and Mammalian Genotyping service (http://research.marshfieldclinic.org/genetics/home/),the polymorphic Range for D9S1876 marker was 132-152 bp, which is equal to alleles 1-9 in this study (Table1). Interestingly, the allele number 9 with 154 bp is a new allele for the D9S1876 which was found in the Iranian population. There was no deviation from HWE in D9S1876 locus, and the p-values were above the expected threshold (P>0.05) in this study. The high degree of PIC values (0.77) introduced this marker as a highly informative polymorphism in the Iranian Population. Data analysis of D9S1837 marker indicated allele 5 with 36.36% frequency was the most frequent allele out of the alleles identified in the Iranian population. Based on the present Study, unlike previously reported alleles in other populations in UniSTS databank, the Absence of 227, 229, 231, 233 and 235 bp alleles in the Iranian population could be clarified. HWE distribution analysis demonstrated no deviation of this marker, which shows the need For further analysis on this marker. Furthermore, the PIC values indicated high informativeness of D9S1837 marker for linkage analysis in this population. D9S1799 polymorphic marker was reported with allele size ranging 139-178 bp in UniSTS Data of Probe database. However, one allele was found in the present investigation (allele 1 With 137 bp), in addition to previously reported alleles. Also, allele 8 with 13.64% frequency Was demonstrated as the most frequent allele out of fifteen identified alleles in the Iranian Population. HWE p-value for this population was above 0.05. Moreover, based on the results Form PIC tests, D9S1799 marker (PIC values>0.7) could be considered as a highly Informative polymorphism in the studied Iranian population. Heterozygosity of all markers Was above 70%, but D9S1799 had the highest heterozygosity (about 89.7%), which shows Effectiveness of this marker in diagnosis. Pair-wise LD test on the adjacent pair of the studied markers was carried out. The data Revealed that D9S1876-D9S1799 had remarkable value (P<0.00001), demonstrating Significant LD in the studied population. On the other hand, LD analysis confirmed presence Of LD for D9S1837-D1799 pair (P=0.0011) in the Iranian population. In this study, estimation of haplotype frequency indicated the ecneteixe of eleven informative Haplotypes in the Iranian population (Table 4); therefore, these eleven common haplotype could be used for improving the quality of molecular screening of TMC1 mutations. These Results strongly support the application of these genetic markers for carrier detection and prenatal diagnosis in families with an affected individual in Iran.

5 Conclusions

The findings of the present study provided, for the first time, the data on D9S1876, D9S1837 and D9S1799 genetic markers located at the TMC1 gene region in the Iranian population. The high level of heterozygosity (0.77-0.89),

appropriate PIC values and the presence of LD Would suggest that these loci could be used as an informative tool in the diagnosis of TMC1-Based ARNSHL. The estimated informative haplotypes would therefore help to optimize Molecular tests for TMC1 in ARNSHL mutation detection linkage-based strategies in Iranian population.

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7 References

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