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Allele Frequency of D12S1632, D12S329, D12S96, D16S3096 and D16S2624 in four Ethnic Groups and Its Relationship With Metabolic Syndrome in Tehran Lipid and Glucose Study

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Background: Variation in drug resistance and susceptibility to various diseases may be related to difference in allele frequencies of the variants at the population level.
Objectives: The present study aimed to investigate the allele frequencies of five short tandem repeats (STR) loci in two different chromosomes of candidates from Tehran lipid and glucose study.
Materials and Methods: For this study, a representative sample of 563 individuals (130 affected by metabolic syndrome) from Tehran, including four different ethnic groups of Iran, was selected. Five STRs including D12S1632, D12S329, D12S96, D16S3096 and D16S2624 were analyzed using the fragment analysis method. Allele frequency, polymorphism information content (PIC) values, observed and expected heterozygosity, discrimination power, matching probability, power of discrimination, power of exclusion and paternity index were calculated for the whole sample.
Results: There was no significant deviation in allelic frequencies from Hardy-Weinberg equilibrium for all the studied markers except for D12S1632 and D12S329. The long alleles in D12S329 were significantly more frequent in patients with metabolic syndrome ($P < 0.05$).
Conclusions: This study revealed allele frequency of some STRs on chromosome 12 and 16 for the first time in Iran, and indicated differences between subjects with metabolic syndrome and subjects in the control group.

Keywords: Chromosome 12; Chromosome 16; Allele Frequency; Ethnic Groups; Iran

1. Background

Analyzing genome variation is the most popular research in the field of genetic. This variation plays an important role in drug response and prediction of the disease (1). The Iranian population consists of around seventy million individuals consisting of people of many religions and ethnic backgrounds cemented by the Persian culture. Ethnic groups include Persians (51%), Azeris (24%), Gilaki and Mazandarani (8%), Kurds (7%), Arabs (3%), Baluchi (2%), Lurs (2%), Turkmens (2%) and others (1%) (2). These ethnic groups may have variations in their DNA sequences. The study of variations in DNA sequence is valuable when it is performed among individuals within the same population or among different populations. Tehran Lipid and Glucose Study (TLGS) is a prospective study of more than 15000 individuals (3-74 years) that live in the 13th district of Tehran Metropolitan. This study aims to develop population-based measures to alter the life-style and prevent the rising trend of non-communicable diseases. Furthermore, TLGS also aims to identify and tackle the risk factors for non-communicable diseases in a representative sample of individuals residing in Tehran, who were recruited by a stratified cluster sampling method (3, 4). Metabolic syndrome is a combination of medical disorders that increases the risk of developing cardiovascular disease and diabetes (5). The prevalence of the metabolic syndrome is 32% in adults (6) and 10% in adolescents (7). In the present study, we aimed to determine the allele frequency of five microsatellite markers on chromosome 12 and 16, and to observe possible differences in the genetic patterns among several ethnic groups. In addition, this study investigated genetic variation between people with and without the metabolic syndrome.

2. Objectives

This article reports primary results in the form of allele frequency distributions and summary statistics of five different autosomal STR loci.

3. Materials and Methods

Population: based on the frequency of metabolic syndrome, a total of 563 individuals aged 3-87 were randomly selected from the TLGS for analyzing the allele frequency of four microsatellite markers on chromosome eight. All subjects answered a questionnaire covering data on demographic factors, smoking habits and other relevant information. Written informed consent was obtained from each subject. The research council of the Endocrine Research Center of the Shahid Beheshti University of Medical Sciences (M.C) approved this study.

3.1. Ethnic Groups

Four ethnic groups were included: Persian (68%), Turk (18.3%), Mazani/Gilaki (8%) and Kurd/Lur (6.2%). To simplify the analysis, the Mazani and Gilaki (people of northern Iran) and the Lur and Kurd (people of western Iran) were combined.

Metabolic syndrome was defined as a cluster of metabolic risk factors for cardiovascular diseases and type 2 diabetes mellitus. Metabolic syndrome X consists of the following complications, excess abdominal fat, atherogenic dyslipidemia, hypertension, hyperglycemia, insulin resistance, a proinflammatory state and a prothrombotic (thrombosis) state (8).

The following phenotypic measurements were obtained for each subject: body mass, body mass index (BMI), height and blood pressure. Blood samples were collected in EDTA containing tubes and serum in tubes without any anticoagulant. After centrifugation for 10 minutes at 3000 rpm, sera were separated and stored at -70°C in 1.5 mL aliquots. Serum glucose, total cholesterol, high-density lipoprotein-cholesterol (HDL-C) and triglyceride levels were measured immediately from fresh sera as described previously (9). Serum HDL-C levels were measured after precipitation of Apo B containing lipoproteins with dextran-magnesium sulfate (10). Low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein (VLDL) concentrations in samples with serum triglyceride levels < 400 mg/dL were calculated using Friedewald's equation, and one fifth of triglyceride level, respectively (11). Coefficients of variation (CV) for total cholesterol, HDL-C and triglyceride measurements were below 5%.

3.2. DNA Analysis

When genomic DNA was extracted by the proteinase K and salting out standard method, buffy coats were separated from the non coagulated blood samples and stored at -70°C until processing (12). The GeneAmp PCR System 9700 (ABI USA) was used to simultaneously amplify the five STRs loci including D12S1632, D12S329, D12S96, D16S3096 and D16S2624. The characteristics of STRs loci are presented in Table 1. Four out of five have dinucleotide repeats and one has a tetranucleotide repeat. Amplification was performed using 100 ng of total genomic DNA in a final volume of 25 L containing 5 pmol of each primer and gold mix of Taq polymerase (ABI USA).

The amplification conditions were as follows: 95˚C for 11 minutes, followed by 30 cycles of 30 seconds at 94˚C, 60 seconds at 55˚C and 40 seconds at 72˚C, and ending with a single 30-minute extension step at 72˚C. Electrophoresis of the amplification products was performed on an ABI 3100 Genetic Analyzer (Applied Biosystems Co.). The raw data were analyzed by the ABI Data Collection Software and GeneMapper 3.2 (Applied Biosystems). For quality control laboratory internal control standards were used.

3.3. Statistical Analysis

Explanatory statistics were used for population characteristics and data are shown as mean ± standard deviation for normally distributed variables and as percentages for categorical variables. Differences between ethnic groups were evaluated by Student's t-test for normally distributed data. The distribution of the triglycerides was skewed, and a comparison was performed using Mann–Whitney's U-test. Analysis of categorical variables was performed by Chi-square and Fisher's exact tests for contingency

Table 1. Characteristics of the Three STRs Loci

Locus	Location	Repeats Unit	Sequence of Primers	Polymorphic Region
D12S96	12q13.13	$[CA]_n$	CCAGTTCAAACCAGTGACCT Labeled with (PET)	201-227
			TCCATCCTTGTGGGCA	
D12S1632	12q13.2	$[TG]_n$	GCCTAATCAAGATGTCACCA Labeled with (VIC)	208-230
			GCTAGGGAGCCAATTCA	
D12S329	12q14.2	$[GT]_n$	AAGCAATCAGCCAGCCCT Labeled with (NED)	143-171
			TGTCAGAACCTAACAACCCAGAAAG	
D16S2624	16q22.3	$[ATCT]_n$	TGAGGCAATTTGTTACAGAGC Labeled with (6-FAM)	130-148
			TAATGTACCTGGTACCAAAAACA	
D16S3096	16q23.1	$[GT]_n$	GATCTGGCTTACGATGATTTCTAAC Labeled with (PET)	199-229
			CCGTGATGATGTCTGCAAC	

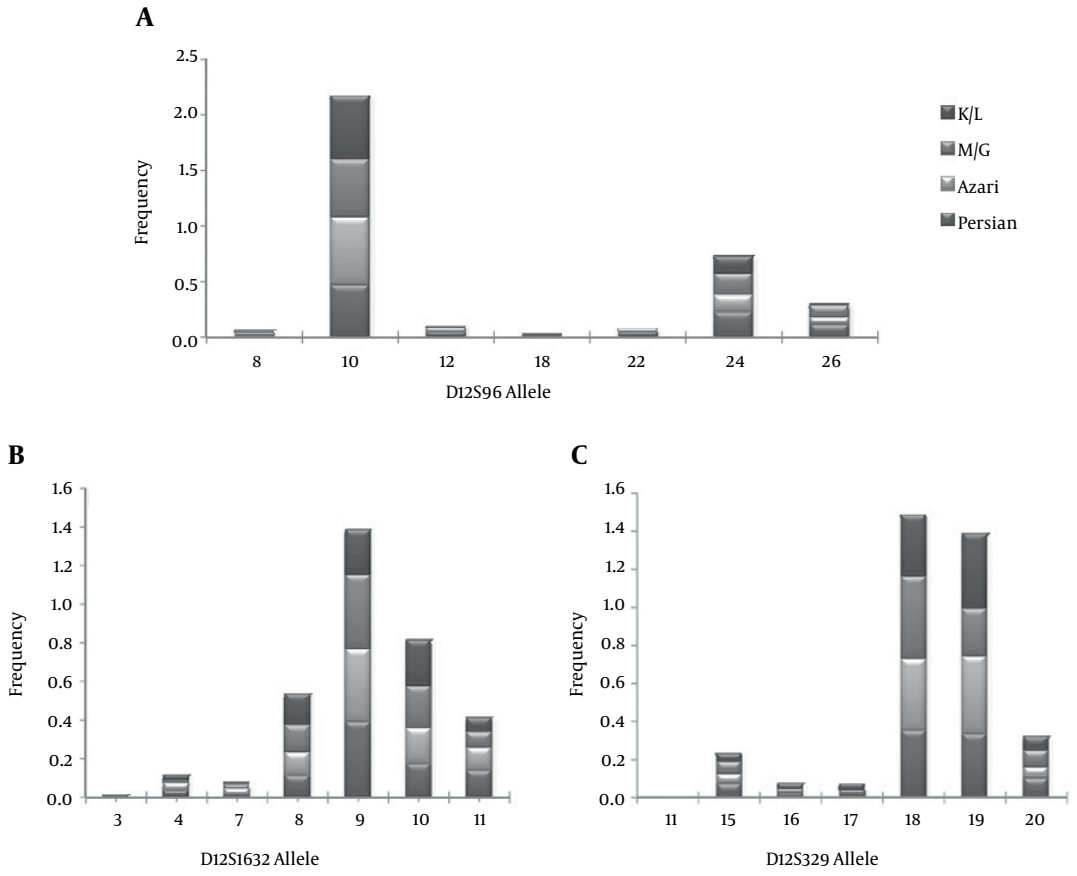
tables. Allele frequency and polymorphic information content (PIC) values were computed by the PowerMarker software (13, 14). Deviation from Hardy-Weinberg equilibrium, as well as observed and expected heterozygosity, were calculated using the GenePop software Version 3.4 (15). The Excel PowerStats spreadsheet from promega (16) was used to calculate discrimination power, matching probability, power of discrimination, power of exclusion and paternity index.

4. Results

The demographic and biochemical parameters of 563 participants consisting of 270 men and 293 women with the mean age of 36 ± 19 are shown in Table 2. There were no statistically significant differences between ethnic groups in biochemical characteristics related to the metabolic syndrome. The allele frequencies for the five STRs loci in 563 unrelated Tehranian samples are presented in Table 3. The most polymorphic marker is D16S2624, this marker has a wide range of size with seven different alleles. Sample populations were observed to be in Hardy-Weinberg Equilibrium (HWE) for all analyzed markers ($P < 0.05$), except for D12S1632 and D12S329. Some factors such as: matching probability, power of discrimination, power of exclusion and paternity index were calculated for this population. Allele frequency distribution in these five microsatellites in four ethnic groups are presented in Figures 1 - 2 the details of the allele frequencies, matching probability, power of discrimination, power of exclusion and paternity index in each ethnic group are presented in Table 4.

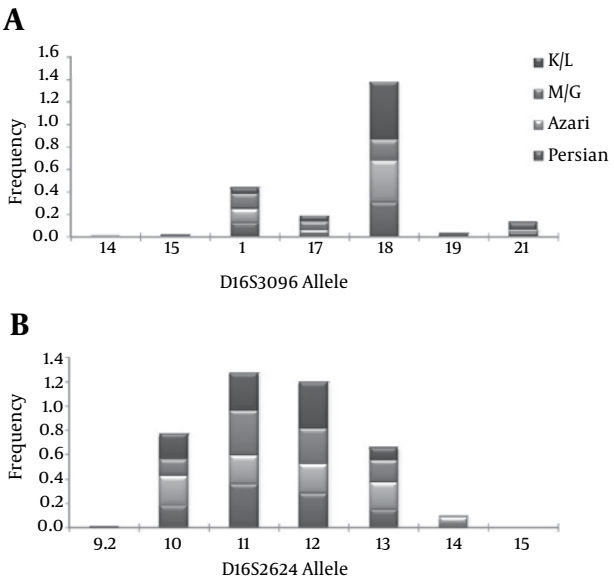
For the D12S96 microsatellite, a total of 11 alleles were observed in the 563 subjects. These were named 201-227 (PCR product length), which correspond to 6-32 (CA)n repeats, respectively. The microsatellite length was used to subdivide samples into two groups according their size: [short (≤ 213), long (> 217)]; [short (≤ 207), medium (207-221) and long (≥ 221)] for case-control analysis. For the D12S1632 microsatellite, a total of 10 alleles were observed in 563 subjects. These were named 208-230 (PCR product length), which corresponded to 4-15 (TG)n repeats, respectively. The microsatellite length was used to subdivide samples into two groups according their size: [short (≤ 220), long (> 220)]; [short (≤ 216), medium (218-222), long (≥ 224)] for case-control analysis. For the D12S329 microsatellite, a total of 10 alleles were observed in 563 subjects. These were named 143-171 (PCR product length),

Figure 1. The Allele Frequency of Microsatellites in Four Ethnic Groups



A) D12S96; B) D12S1632; C) D12S329.

Figure 2. The Allele Frequency of Microsatellites in Four Ethnic Groups



A) D16S3096; B) D16S2624.

which corresponded to 11-25 (GT)n repeats, respectively. The microsatellite length was used to subdivide samples into two groups according their size: [short (≤ 157), long (> 159)]; [short (≤ 153), medium (155-161), long (≥ 163)] for case-control analysis. For the D16S2624 microsatellite, a total of six alleles were observed in 563 subjects. These were named 130-148 (PCR product length), which corresponded to 9.2-14 (ATCT)n repeats, respectively. The microsatellite length was used to subdivide samples into two groups according their size: [short (≤ 136), long (> 140)]; [short (≤ 136), medium (136-140), long (≥ 140)] for case-control analysis. Finally, for the D16S3096 microsatellite, a total of 17 alleles were observed in 563 subjects. These were named 199-229 (PCR product length), which corresponded to 14-29 (GT)n repeats, respectively. The microsatellite length was used to subdivide samples into two groups according their size: [short (≤ 215), long (> 216)]; [short (≤ 209), medium (209-219), long (≥ 219)] for case-control analysis. The allele frequencies of the control and the metabolic syndrome groups were compared for the four microsatellites subdivided in three groups (short, medium and long). In the D12S329, the frequency of long alleles in subjects with metabolic syndrome was significantly higher than the controls ($P < 0.05$) (Table 5). In Table 6 the allele frequencies of the four different ethnic groups were compared for the five microsatellites in two subdivided groups (short and long) and were significantly different.

Table 2. Demographic and Biochemical Parameters of 563 Participants According to Ethnic Groups [a]

Characteristic	Total (n = 463)	Persian (n = 380)	Turk (n = 103)	Mazani/Gilaki (n = 45)	Kurd/Lur (n = 35)
Metabolic syndrome	130 (28)	82 (21.5)	23 (22.3)	11 (24.5)	14 (40)
Age, y	36 ± 19	35 ± 19	37 ± 19	38 ± 18	39 ± 20
Sex, females, %	52	51.3	49.5	53.3	68.6
BMI, kg/m^2					
Women	25 ± 6	26 ± 6	26 ± 5	27 ± 7	26 ± 6
Men	25 ± 5	25 ± 5	25 ± 5	25 ± 5	29 ± 5
Family history of diabetes, %	7.3	8.0	3.9	8.9	8.8
Components of metabolic syndrome					
Waist circumference, cm					
Women	83 ± 15	83 ± 15	84 ± 16	86 ± 15	84 ± 16
Men	90 ± 15	89 ± 15	90 ± 13	88 ± 14	100 ± 12
Fasting plasma glucose, mg/dL	95 ± 29	95 ± 30	92 ± 19	100 ± 37	93 ± 19
Elevated blood pressure, mmHg					
Systolic	113 ± 29	113 ± 20	113 ± 19	112 ± 20	117 ± 25
Diastolic	71 ± 11	70 ± 10	69 ± 11	70 ± 10	74 ± 13
Serum triglycerides, mg/dL	140 ± 87	137 ± 79	143 ± 90	154 ± 130	148 ± 83
HDL cholesterol, mg/dL					
Women	47 ± 1	47 ± 11	46 ± 11	48 ± 12	47 ± 12
Men	41 ± 9	41 ± 9	40 ± 9	473 ± 12	35 ± 7

[a] Data are presented as Means ± SD, No. (%) or %.

Table 3. Allele Frequencies for Five STRs Loci in 563 Unrelated Tehranian Samples [a]

Repeat	D16S2624	D16S3096	D12S1632	D12S329	D12S96
2			0.0009		
3			0.0009		
4			0.0333		
6					0.0026
7			0.0144		
8			0.1180		0.0212
9			0.3793		
9.2	0.0111				
10	0.1908		0.1811		0.5053
11	0.3228		0.1252	0.0017	
12	0.2785		0.1144		0.0362
13	0.1695		0.0162		
14	0.0256	0.0045	0.0072		
15	0.0017	0.0009	0.0090	0.0693	
16		0.1203		0.0197	
17		0.0305		0.0094	
18		0.3142		0.3622	0.0097
19		0.0171		0.3416	
20		0.0018		0.0873	
21		0.0296		0.0771	
22		0.0180		0.0291	0.0362
22.1		0.0036			
23		0.0637			
24		0.1059			0.2046
25		0.2621		0.0026	
26		0.0108			0.0935
27		0.0108			
28		0.0036			0.0309
29		0.0027			
30					0.0556
32					0.0044
PIC	0.7100	0.7750	0.7525	0.6918	0.6558
Ho	0.8395	0.8072	0.7900	0.7736	0.6474
He	0.7523	0.7986	0.7740	0.7370	0.6859
MP	0.1174	0.0728	0.0778	0.1170	0.1393
PD	0.8826	0.9272	0.9222	0.8830	0.8607
PE	0.6584	0.6341	0.5693	0.5365	0.3539
PI	2.9646	2.7574	2.3125	2.1314	1.4246
P	0.0060	0.0000	0.8800	0.9850	0.0000

[a] Abbreviations: H_{obs}, Observed heterozygosity; H_{exp}, expected heterozygosity; MP, matching probability; PIC, Polymorphic information content; P, probability value of exact tests of Hardy-Weinberg disequilibrium; PD, discrimination power; PE, power of exclusion; PI, paternity index.

Table 4. Details of Allele Frequencies in Four Ethnic Groups [a]

	Persian	Azeri	Mazani/ Gilaki	Kurd/Lur
D12S96				
MP	0.133	0.202	0.152	0.189
PD	0.867	0.798	0.848	0.811
PIC	0.675	0.573	0.640	0.579
PE	0.354	0.270	0.422	0.412
PI	1.43	1.20	1.64	1.61
Allele Frequencies				
Homozygotes, %	35.1	41.7	30.4	31.0
Heterozygotes, %	64.9	58.3	69.6	69.0
Total Alleles	724	206	92	58
D12S329				
MP	0.116	0.149	0.105	0.157
PD	0.884	0.851	0.895	0.843
PIC	0.703	0.633	0.692	0.681
PE	0.551	0.453	0.529	0.643
PI	2.21	1.76	2.09	2.83
Allele Frequencies				
Homozygotes, %	22.7	28.4	23.9	17.6
Heterozygotes, %	77.3	71.6	76.1	82.4
Total Alleles	750	204	92	68
D12S1632				
MP	0.084	0.079	0.116	0.090
PD	0.916	0.921	0.884	0.910
PIC	0.743	0.761	0.738	0.791
PE	0.586	0.532	0.500	0.588
PI	2.41	2.11	1.95	2.43
Allele Frequencies				
Homozygotes, %	20.7	23.7	25.6	20.6
Heterozygotes, %	79.3	76.3	74.4	79.4
Total Alleles	714	194	86	68
D16S2624				
MP	0.126	0.108	0.145	0.168
PD	0.874	0.892	0.855	0.832
PIC	0.695	0.739	0.674	0.651
PE	0.643	0.779	0.529	0.588
PI	2.83	4.64	2.09	2.43
Allele Frequencies				
Homozygotes, %	17.7	10.8	23.9	20.6
Heterozygotes, %	82.3	89.2	76.1	79.4
Total Alleles	758	204	92	68
D16S3096				
MP	0.076	0.083	0.111	0.130
PD	0.924	0.917	0.889	0.870
PIC	0.774	0.753	0.719	0.666
PE	0.670	0.531	0.591	0.510
PI	3.08	2.10	2.44	2.00
Allele Frequencies				
Homozygotes,%	16.2	23.8	20.5	25.0
Heterozygotes, %	83.8	76.2	79.5	75.0
Total Alleles	714	202	88	56

[a] Abbreviations: MP, matching probability; PIC, Polymorphic information content; PD, discrimination power; PD, power of discrimination; PE, power of exclusion; PI, paternity index.

Table 5. Allele Frequencies of People with and Without Metabolic Syndrome

	Non Metabolic Syndrome	Metabolic Syndrome
D12S96		
Short allele	0.5581 (480)[a]	0.5977 (159)
Medium allele	0.2465 (212)	0.2481 (66)
Long allele	0.1953 (168)	0.1541 (41)
D12S329		
Short allele	0.0887 (79)	0.0919 (25)
Medium allele	0.8146 (725)[b]	0.7610 (207)
Long allele	0.0966 (86)	0.1470 (40)
D12S1632		
Short allele	0.0305 (26)	0.0465 (12)
Medium allele	0.5176 (441)	0.4961 (128)
Long allele	0.4518 (385)	0.4573 (118)
D16S3096		
Short allele	0.1662 (140)	0.1893 (50)
Medium allele	0.2220 (187)	0.2234 (59)
Long allele	0.6116 (515)	0.5871 (155)
D16S2624		
Short allele	0.5339 (441)	0.5233 (135)
Medium allele	0.2772 (229)	0.2752 (71)
Long allele	0.1889 (156)	0.2016 (52)

[a] Number in parenthesis denote number of subjects
[b] Long allele frequency *vs.* medium allele frequency, significance at $P < 0.001$ by the Chi-square test.

Table 6. Allele Frequencies in Four Ethnic Groups

Marker	Persian	Azeri	Mazani/ Gilaki	Kurd/Lur
D12S96[a]				
Short allele	0.5360 (387)[b]	0.6747 (139)	0.5888 (53)	0.5689 (33)
Long allele	0.4639 (335)	0.3252 (67)	0.4111 (37)	0.4310 (25)
D12S329				
Short allele	0.4558 (341)	0.4460 (91)	0.5333 (48)	0.4264 (29)
Long allele	0.5441 (407)	0.5539 (113)	0.4666 (42)	0.5735 (39)
D12S1632				
Short allele	0.5407 (385)	0.5721 (111)[c]	0.5714 (48)	0.4411 (30)
Long allele	0.4592 (327)	0.4278 (83)	0.4285 (36)	0.5588 (38)
D16S3096				
Short allele	0.4747 (338)[d]	0.5396 (109)[e]	0.4069 (35)[f]	0.6250 (35)
Long allele	0.5252 (374)	0.4603 (93)	0.5930 (51)	0.3750 (21)
D16S2624				
Short allele	0.5489 (415)[g]	0.4754 (97)	0.5222 (47)	0.5147 (35)
Long allele	0.4510 (341)	0.5245 (107)	0.4777 (43)	0.4852 (33)

[a] Number in parenthesis denote number of subjects.
[b] Persian allele frequency vs. Azeri allele frequency, significance at $P < 0.001$ by the Chi-square test.
[c] Azeri allele frequency vs. Kurd/Lur allele frequency, significance at $P < 0.001$ by the Chi-square test.
[d] Persian allele frequency vs. Kurd/Lur allele frequency, significance at $P < 0.001$ by the Chi-square test.
[e] Azeri allele frequency vs. Mazani/Gilaki allele frequency, significance at $P < 0.001$ by the Chi-square test.
[f] Kurd/Lur allele frequency vs. Mazani/Gilaki allele frequency, significance at $P < 0.001$ by the Chi-square test.
[g] Persian allele frequency vs. Azeri frequency, significance at $P < 0.001$ by the Chi-square test.

5. Discussion

This study is the first allele frequency report related to chromosomes 12 and 16 in the Iranian population. Based on our knowledge there has been no allele frequency data for our selected microsatellite on chromosome 12. To confirm the new allele in D16S2624, the ALFERED database was accessed. Furthermore, in subjects with metabolic syndrome, the long alleles were significantly more frequent in D12S329 ($P < 0.05$). Between the different ethnic groups there were some differences in short and long allele frequencies. D16S2624 is a tetra nucleotide repeat marker but in this population one new allele (15.2) was seen. The most heterozygote marker in the total population and in different ethnic groups was D16S2624. The power of discrimination ranged from a minimum of 0.798 for D12S96 locus in the Azeri group to a maximum of 0.924 for D16S3096 locus in the Persian group. In the Iranian population, the distribution of the analyzed loci alleles was not previously studied. The present dataset will add to the reference database and will be helpful in population genetics and diversity studies. Differences between medium and long allele frequencies in D12S329 of subjects with metabolic syndrome in comparison with controls may be a sign of association between this region and the presence of the metabolic syndrome. Further analysis with more microsatellites in this region can lead us to the genetic cause of metabolic syndrome. Ethnic groups have some variation in allele frequency but the sample size was not big enough to reach a comprehensive conclusion. In the future the most important markers in this population have to be checked in order to improve knowledge regarding the genetic pattern of the Iranian population.

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Authors' Contributions

Study concept and design: Daneshpour, Azizi, Houshmand and Hedayati. Acquisition of data: Azizi and Daneshpour. Analysis and interpretation of data: Daneshpour, Zeinali, Houshmand, Alfadhli, Zarkesh. Drafting of the manuscript: Daneshpour, Zarkesh, Hedayati and Zeinali. Critical revision of the manuscript for important intellectual content: Azizi, Alfadhli. Statistical analysis: Daneshpour, Zarkesh and Zeinali. Administrative, technical, and material support: Azizi and Alfadhli. Study supervision: Daneshpour, Azizi, Houshman and Zeinali.

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