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Genetic variations of 21 STR markers on chromosomes 13, 18, 21, X, and Y in the south Iranian population

J. Saberzadeh[1], M.R. Miri[1], M.B. Tabei[2,3,4], M. Dianatpour[2,3,4] and M. Fardaei[2,3,4]

[1]Medical Biotechnology Department, School of Advanced Medical Sciences and Technology, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of Medical Genetics, School of Medical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[4]Comprehensive Medical Genetic Center, Shiraz University of Medical Sciences, Shiraz, Iran

Corresponding author: M. Fardaei
E-mail: mfardaei@sums.ac.ir

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ABSTRACT. Quantitative fluorescent polymerase chain reaction (QF-PCR), in recent years, has been accepted as a rapid, high throughput, and sensitive method for prenatal diagnosis of common chromosomal aneuploidies. Since short tandem repeats (STRs) are the cornerstone of QF-PCR technique, selection of the most polymorphic STR markers is an essential step for a successful QF-PCR assay. The genetic variation parameters of each STR marker differ among different populations. In this study, we investigated the size, frequency, heterozygosity,

polymorphism information content, power of discrimination, and other genetic polymorphism data for 21 STR markers on chromosomes 13, 18, 21, X, and Y in 1000 amniotic fluid samples obtained from south Iranian women. Our results showed that all the 21 STR markers are highly polymorphic and informative in our population. The heterozygosity, polymorphism information content, and power of discrimination of the markers were 62-91.1%, 0.61-0.91, and 0.830-0.976, respectively. The locus D18S386 was the most polymorphic STR, while the locus DXYS218 was the least polymorphic STR among all the studied STRs. The present study has provided extensive data regarding the efficiency of the 21 STR markers for diagnosis of chromosomes 13, 18, 21, X, and Y aneuploidies in the south Iranian population.

Key words: Short tandem repeats; Prenatal diagnosis; Quantitative fluorescent polymerase chain reaction; Iranian population

INTRODUCTION

The most common prenatally diagnosed abnormalities are aneuploidies such as trisomy 21 (Down syndrome), trisomy 18 (Edward syndrome), trisomy 13 (Patau syndrome), and sex chromosome aneuploidies (Divane et al., 1994). It has been estimated that 5% of all human conceptions are aneuploidy (Hassold and Hunt, 2001). Despite the efficiency of karyotyping as a gold standard technique (Shaffer and Bui, 2007), quantitative fluorescent polymerase chain reaction (QF-PCR) has emerged as a rapid, accurate, cost effective, and high throughput method for prenatal diagnosis of chromosomal aneuploidies (Cirigliano et al., 2006; Mann et al., 2008; Mann and Ogilvie, 2012; Rostami et al., 2015). QF-PCR is a PCR-based technique that amplifies short tandem repeats (STRs) located on chromosomes of interest to determine the copy numbers of those chromosomes present per cell (Langlois and Duncan, 2011). STR markers are highly polymorphic repetitive sequences distributed throughout the genome of eukaryotes with a low mutation rate (Tóth et al., 2000). These abovementioned characteristics explain the wide application of STRs in forensic individual identification, prenatal and postnatal diagnosis of chromosome aneuploidies, and paternity testing (Mansfield, 1993). STR markers demonstrating two peaks are heterozygote and are known as informative markers and those demonstrating only one peak are homozygote and are known as uninformative markers (Andonova et al., 2004; Quaife et al., 2004). In order to perform a reliable and accurate QF-PCR assay, a minimum of two heterozygote markers per chromosome are required to confirm the copy number of the chromosome of interest (Association for Clinical Cytogenetics and Clinical Molecular Genetics Society, 2012). Consanguineous marriages result in decreased heterozygosity and informativeness of STR markers (Choueiri et al., 2006). Hence, for populations with a higher degree of consanguinity, evaluating the polymorphisms and heterozygosity of STRs is an essential step before applying QF-PCR (Lee et al., 2010; Jain et al., 2012). The aims of this study were to evaluate the genetic variation parameters of 21 STR markers for detection of common chromosomal aneuploidies in the south Iranian population. Here, we represented the genetic variations and population data of 1000 amniotic fluid samples and compared our findings with those obtained for data from other populations. To the best of our knowledge, no data for these STR markers are currently available for our population.

MATERIAL AND METHODS

Sample collection

Amniotic fluid (AF) samples (N = 1000) were collected from women, at an increased risk of chromosomal abnormalities, in the first or second trimester screening programs referred to Comprehensive Medical Genetic Center (Shiraz, Iran) from March 2014 to June 2015. Informed consent for sample collection and consequent analysis was obtained from all contributors. The samples were divided into two parts, for genomic extraction followed by QF-PCR and cytogenetic diagnosis (karyotyping) as a confirmatory test.

DNA extraction

The "salting out protocol" was carried out according to the manufacturer's guidelines (RIBO-prep, Moscow, Russia). Briefly, 1 to 2 mL AF sample was centrifuged (Eppendorf, Sigma, St. Louis, MO, USA) at 10,416 *g* for 10 min. After discarding the supernatant, 100 µL prepared samples were mixed thoroughly with 300 µL solution for lysis by vortexing. The tubes were centrifuged for additional 5 s to ensure that there were no residual drops left on the cap; then, the samples were incubated in a 65°C water bath for 5 min. Precipitation solution (400 µL) was added and mixed with the samples by vortexing. The samples were centrifuged at 14,549 *g* in a bench top centrifuge for 5 min, and the supernatant was discarded carefully without disturbing the pellet. After pellet washing steps, all the tubes were incubated at 65°C for 5 min with open caps (to dry the pellet). Buffer (30 µL) was added to each tube and mixed with the pellet by vortexing, followed by incubation at 65°C for 5 min with occasionally stirring by vortex. Concentration of the extracted DNA was determined by ultraviolet absorbance measurement at 260 nm using the NanoDrop Lite spectrophotometer (Thermo Scientific, USA).

PCR

Devyser Extend v2 (Hägersten, Sweden) aneuploidy detection kit was used for the amplification of 21 STR markers on chromosomes 13, 18, 21, X, and Y in each tube. In general, PCR amplification was carried out in a 25-µL total volume by adding 20 µL prepared PCR mix containing Hot Start Taq polymerase to the extracted genomic DNA (3-30 ng). After activation of the enzyme at 95°C for 15 min, 27 cycles were applied according to the manufacturer instructions using Veriti thermal cycler (Applied Biosystems, Foster City, CA, USA).

Capillary electrophoresis

Fragment analysis of the PCR products were performed by Applied Biosystems 3500 Genetic Analyzer (Applied Biosystems), using the 3500 Data Collection software, 50 cm capillary array length, and Performance Optimized Polymer 7 (Applied Biosystems) for electrophoresis. In brief, each amplified sample (1.5 µL) was added to 15 µL ultrapure Hi-Di formamide (Applied Biosystems) and 0.3 µL size standard LIZ 500 (GeneScan, Applied Biosystems) on a MicroAmp optical 96-well reaction plate (Applied Biosystems). Prior to electrophoresis, the mixture was heated for 5 min at 95°C for denaturation. Data were analyzed and electropherograms were made using the Gene Mapper ID software v3.2.

Genetic variation analysis

Heterozygosity, typical paternity index, polymorphism information content (PIC), power of exclusion (PE), probability of identity (matching probability), and power of discrimination (PD) were calculated using the PowerStatsv12 software (Promega Corp., Madison, WI, USA).

RESULTS

Allelic size

The study was performed on 1000 AF samples. The observed allelic sizes for each STR marker are shown in Table 1. Minor differences can be seen between the observed and expected allelic sizes, and most of the observed allelic sizes were narrower than the expected sizes.

Table 1. Observed allelic sizes of the STR markers included in this study compared with those reported by the commercial kit manufacturer.

Marker	Location	Expected size (bp)	Observed size (bp)	Observed alleles (bp)
D18S978	18q12.3	195-230	204-228	204, 208, 212, 216, 220, 224, 228
D18S535	18q12.3	300-350	309-337	309, 313, 317, 321, 325, 329, 333, 337
D18S386	18q22.1	338-430	344-412	344, 352, 356, 360, 364, 368, 372, 376, 380, 384, 388, 392, 396, 400, 404, 408, 412
D18S976	18p11.31	440-495	448-476	448, 450, 452, 454, 458, 460, 462, 464, 466, 468, 472, 476
GATA178F	18p11.32	350-410	368-396	368, 370, 372, 376, 380, 384, 388, 392, 396
D13S742	13q12.12	222-334	235-295	235, 249, 253, 255, 257, 261, 265, 269, 271, 273, 275, 279, 283, 285, 295
D13S634	13q21.32- q21.33	365-435	392-416	392, 396, 400, 402, 404, 406, 408, 410, 412, 414, 416
D13S628	13q31.1	420-475	432-468	432, 440, 444, 448, 452, 456, 460, 464, 468
D13S305	13q13.3	435-505	436-476	436, 444, 448, 452, 456, 460, 464, 468, 472, 476
D13S1492	13q21.1	100-175	104-160	104, 112, 116, 120, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160
D21S1435	21q21.3	150-208	171-195	171, 175, 179, 183, 187, 191, 195
D21S1411	21q22.3	245-345	261-335	261, 275, 285, 289, 293, 297, 301, 305, 307, 309, 311, 313, 315, 317, 319, 323, 327, 331, 335
D21S1444	21q22.13	440-495	455-483	455, 459, 463, 467, 471, 475, 479, 483
D21S1442	21q21.3	362-420	373-405	373, 377, 381, 385, 389, 393, 397, 401, 405
D21S1437	21q21.1	105-152	113-149	113, 117, 121, 125, 129, 133, 137, 141, 145, 149
D21S11	21q21.1	215-290	235-269	235, 239, 243, 247, 251, 253, 257, 261, 265, 269
DXS1187	Xq26.2	120-170	131-155	131, 133, 135, 139, 143, 147, 151, 155
XHPRT	Xq26.2-q26.3	265-308	282-302	282, 286, 290, 294, 298, 302
DXS2390	Xq27.1-q27.2	312-357	319-347	319, 323, 327, 331, 335, 339, 343, 347
DXYS267	Xq21.31, Yp11. 31	175-217	183-203	183, 187, 191, 195, 199, 203
DXYS218	Xp22.33, Yp11.32	215-260	219-255	219, 227, 231, 235, 239, 243, 247, 251, 255

Allelic frequency

The frequency of each allele was calculated for all 21 STR markers and the results are shown in Figure 1 and **Tables S1** and **S2**. The most frequent alleles in our population for D13S742 marker were 257-bp (16%), 261-bp (14%), and 269-bp (13%) alleles; D13S634 marker, 400-bp allele (19%); D13S628 marker, 456-bp allele (39%); D13S305 marker, 468-bp allele (27%); and for D13S1492 marker, 136-bp allele (19%) (Figure 1A). As shown in Figure 1B, the most frequent alleles for D18S535 marker were 321- and 329-bp alleles (27%); D18S978 marker, 212-bp (34%) and 216-bp (29%) alleles; D18S386 marker, 368-bp (15%) and 396-bp (12%) alleles; D18S976 marker, 458-bp allele (33%); and for GATA178F marker, 380-bp allele (25%). As shown in Figure 1C, the most frequent allele for D21S1435 marker

was 183-bp allele (39%); D21S1411 marker, 301-bp (18%), 297-bp (15%), and 305-bp (13%) alleles; D21S1444 marker, 467-bp allele (33%); D21S1442 marker, 389-bp allele (24%); D21S1437 marker, 133-bp allele (33%); and for D21S11 marker, was 247-bp allele (26%). As shown in Figure 1D, the most frequent allele for DXS1187 marker was 147-bp allele (29%); XHPRT marker, 286- and 290-bp alleles (34%); DXS2390 marker, 335-bp allele (29%); for DXS267 marker, 195-bp allele (46%); and for DXS218 marker, 243-bp allele (46%).

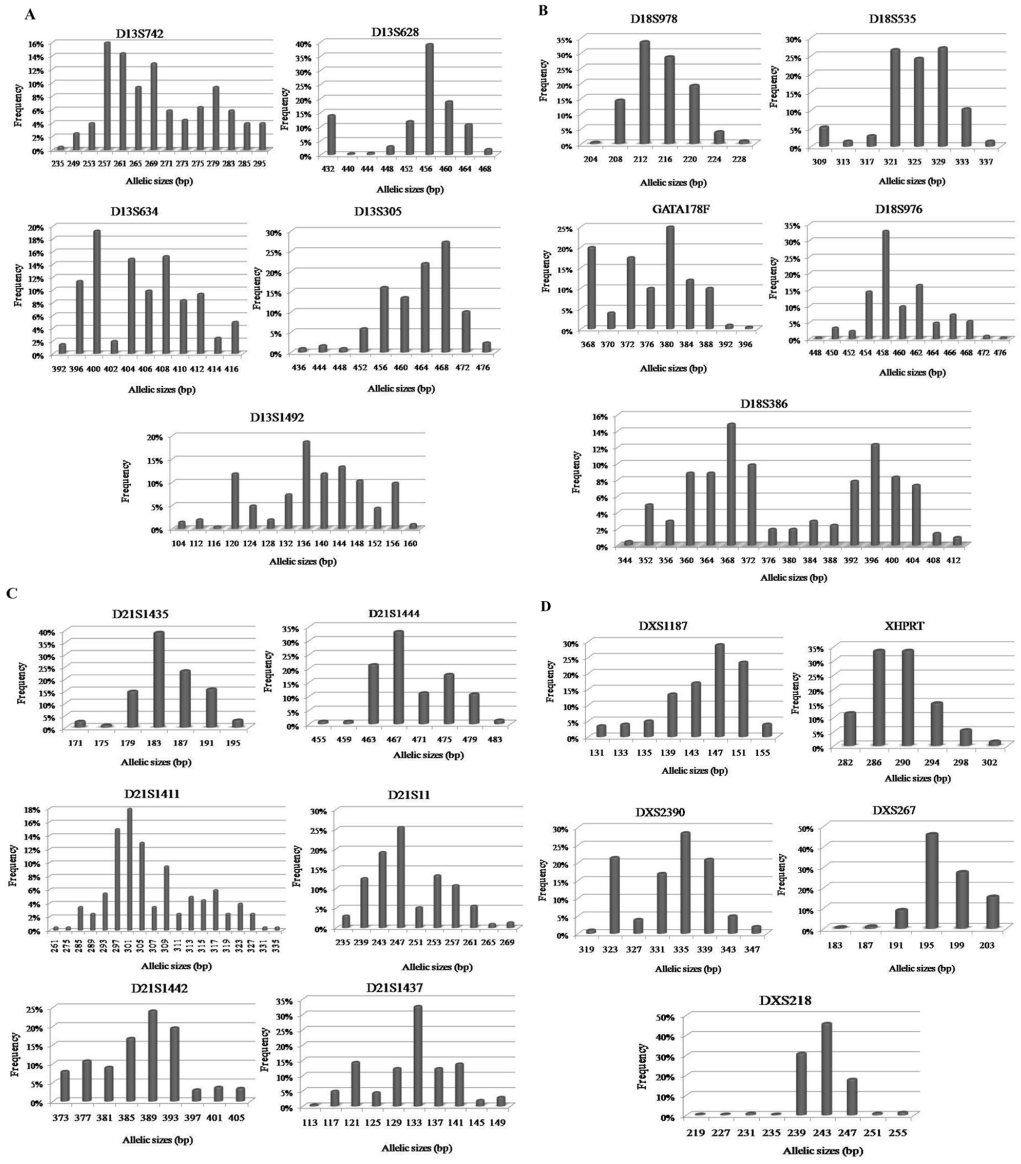

Figure 1. Allelic sizes and frequencies. A total of 1000 samples were evaluated and the size and the frequency of each allele were calculated for all 21 STR markers on the chromosomes: **A.** 13, **B.** 18, **C.** 21, and **D.** X and Y.

Genetic variation parameters

In this study, heterozygosity and other genetic variation parameters were calculated for evaluating the polymorphism quality of 21 STR markers (Table 2). All 21 STR markers displayed acceptable polymorphism and heterozygosity in our population. Heterozygosity, PIC, and PD were 62-91.1%, 0.61-0.91, and 0.830-0.976, respectively. Among all the STR markers, D18S386, D13S742, D21S1411, D21S1437, D21S11, D13S305, and D13S1492 showed the highest heterozygosity of more than 80%, with heterozygosity of 91.1, 89.1, 88, 88, 84.2, 83.2, and 81.8%, respectively. DXYS218, DXYS267, and D21S1435 STRs showed the least heterozygosity, with heterozygosity of 62, 70, and 70.1%. According to the results obtained for heterozygosity and PIC of each of the 21 STR markers, the locus D18S386 was the most polymorphic marker and DXYS218 was the least polymorphic marker.

Table 2. Genetic variation parameters of the 21 STRs on chromosomes 13, 18, 21, X, and Y in the south Iranian population. Chromosome X markers were evaluated in 1000 samples collected from women.

Locus	Heterozygosity %	Typical paternity index	Polymorphism information content (PIC)	Power of exclusion (PE)	Power of discrimination (PD)	Probability of identity
D18S978	74.3	1.94	0.7	0.497	0.879	0.121
D18S535	78.2	2.3	0.75	0.566	0.911	0.089
D18S386	91.1	5.61	0.91	0.818	0.971	0.029
D18S976	76	2.08	0.8	0.527	0.937	0.063
GATA178F	80.4	2.55	0.78	0.607	0.929	0.071
D13S742	89.1	4.59	0.90	0.777	0.976	0.024
D13S634	79	2.38	0.91	0.581	0.975	0.025
D13S628	76.2	2.1	0.73	0.531	0.912	0.088
D13S305	83.2	2.47	0.81	0.659	0.938	0.062
D13S1492	81.8	2.75	0.88	0.633	0.970	0.030
D21S1435	70.1	1.66	0.72	0.427	0.906	0.094
D21S1411	88	4.17	0.89	0.755	0.973	0.027
D21S1444	74	1.93	0.76	0.495	0.923	0.077
D21S1442	80.4	2.55	0.83	0.607	0.950	0.050
D21S1437	88	4.21	0.8	0.757	0.936	0.064
D21S11	84.2	3.16	0.83	0.678	0.952	0.048
DXS1187	78	2.27	0.78	0.562	0.929	0.071
XHPRT	72	1.79	0.7	0.460	0.885	0.115
DXS2390	79	2.38	0.78	0.581	0.927	0.073
DXYS267	70	1.67	0.63	0.428	0.833	0.167
DXYS218	62	1.32	0.6	0.316	0.83	0.170

DISCUSSION

The QF-PCR assay is a widely used clinical assay owing to its high sensitivity and specificity and is now performed routinely in the majority of prenatal centers worldwide (Andonova et al., 2004; Onay et al., 2008; Putzova et al., 2008). In order to achieve conclusive results in a QF-PCR aneuploidy detection assay, selection of the most heterogeneous STR markers is the key step. Since the informativeness of STR markers is a population-dependent factor, it is recommended that markers should be evaluated for a particular population. Aneufast (Wollerau, Switzerland), ChromoQuant (Solna, Sweden), and Devyser are among the most popular QF-PCR aneuploidy detection kits for prenatal diagnosis of aneuploidies. A limitation of the aforementioned kits is that these are designed for populations of European-descent; hence, they may be inefficient for other populations. There have been studies that evaluated the efficiency of Aneufast and ChromoQuant kits and assessed the STR markers they employed

(Cirigliano et al., 2009; Nasiri et al., 2011), but no publication that has evaluated all the STR markers applied in Devyser QF-PCR kit. In this study, we validated all the STR markers used in the Devyser commercial kit and could provide extensive data on them due to the sample size of 1000. Compared to the results obtained in studies by Mann et al. (2001, 2008, 2012), our results showed major heterozygosity differences between UK and Iranian study population. The heterozygosity of D18S535, D13S634, D21S1435, D21S1411, D21S1437, D21S11, XHPRT, and DXYS267 markers in the south Iranian population was lower than that in study populations reported by Mann et al. (2001, 2012) (Table 3). The heterozygosity reported by Mann et al. (2001, 2012) for D18S978, D18S386, D13S742, D13S628, D13S305, and DXS1187 markers was lower than that observed in the south Iranian population (Table 3). Interestingly, some previously mentioned markers (D18S535, D21S1411 and D21S11, D18S978, D13S742, D13S628, and D13S305) showed heterozygosity results similar to those reported by Moftah et al. (2013) in Germany (Table 3). The heterozygosity of D18S535 and D21S1411 markers was similar to that observed in the Lebanese population (Choueiri et al., 2006) and the heterozygosity of D18S535, D13S634, and D21S11 was similar to that reported by Bili et al. (2002) in the Greek population (Table 3). Our findings showed that the heterozygosity rates of STR markers are different for different populations. Although we found that all 21 STR markers included in this study possess an acceptable rate of informativeness, the number of STR markers was limited, and there might be STRs with higher heterozygosity and polymorphisms in our population. This study is the first report on genetic variation data for the 21 STR markers in the south Iranian population.

Table 3. STR heterozygosity comparison of our study with other studies indifferent populations.

Locus	Present study (South of Iran)	Mann et al. (UK)	Moftah et al. (Germany)	Choueiri et al. (Lebanon)	Billi et al. (Greece)
D18S535	78.2	92	79.5	80.2	76
D13S634	79.0	81.2	75.3	-	80
D21S1435	69.9	75	55.7	77	-
D21S1411	90.2	93.3	90.4	89.1	83
D21S1437	66.4	84	-	-	-
D21S11	84.2	90	81.9	78.9	83
D21S1444	74.1	-	-	-	85
XHPRT	72	78	-	-	80
DXYS267	70	87	-	-	-
D18S978	74.3	66.7	73.8	-	-
D18S386	91.1	87.5	87.7	-	-
D18S976	76	-	-	82.8	-
D13S742	89.1	75	92	-	-
D13S628	76.2	68.8	73.3	-	-
D13S305	83.2	75	85.3	-	-
DXS1187	78	72	-	-	-

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

Table S1. Heterozygosity, size, and frequency of the most frequent allele for each STR marker.

Table S2. Frequencies of different alleles for the 21 STR markers on chromosomes 13, 18, 21, X and Y.