Application of Molecular Cytogenetic Technique for Rapid Prenatal Diagnosis of Aneuploidies in Iranian Population

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Abstract

Objective: Classic cell culture and karyotyping is routinely used for prenatal detection of different chromosomal abnormalities. Molecular cytogenetic techniques have also recently been developed and used for this purpose. Quantitative florescence PCR using short tandem repeat (STR) markers has more potential for high throughput diagnosis. Marker heterozygosity in short tandem repeats (STR) is of critical importance in the clinical applicablity of this method.

Materials and Methods: Different STR markers on chromosomes 13, 18, 21, X and Y were analysed from amniotic samples to detect related disorders such as Down, Edward, Patau, Klinefelter sundromes , as well as sex chromosomes numerical abnormalities .

Results: In our population some markers (D18S976, DXS6854, D21S11, and D21S1411) showed alleles with sizes out of expected ranges. But others occupied narrower range of predicted distribution. Most markers have enough heterozygosity (66.3-94.7) to be used for prenatal diagnosis. Furthermore, results obtained from full karyotype for all samples were in concordance with results of molecular cytogenetic testing.

Conclusion: It is concluded that, in urgent situations, if proper markers used, molecular cytogenetic testing (QF-PCR) could be a useful method for rapid prenatal diagnosis (PND) in populations with high rate of consanguinity such as Iran.

Keywords: Prenatal diagnosis, Chromosomal aberration, Short tandem repeat, Heterozygosity, Iranian population

Introduction

Down syndrome and other numerical chromosomal aberrations are among the most frequent causes of mental retardation and developmental problems (1). Various first and second trimester screening programs and invasive diagnostic tests have been developed for prenatal approach to these conditions (2). If diagnostic tests required, the whole process, from sampling to final report, takes at least two weeks (3). In recent years, there have been a growing interest among geneticists to use rapid molecular cytogenetic techniques such as fluorescent in situ hybridization (FISH), multiplex ligation dependent probe amplification(MLPA)and quantitative fluorescence polymerase chain reaction

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Table 1 : Characteristics and detected heterozygosity for multiplex1 markers in Iranian population

	AMEL	D18S391	D18S976	XHPRT	D13S742	D18S386	D13S634	D13S628	D13S305	D18S535
Location	Xp22.31-Xp22.1 Yp11.2	18p11.31	18p11.31	Xq26.1	13q12.13	18q22.1	13q21.33	13q31.1	13q13.3	18q12.3
Expected Size (bp)	X:103-108 Y109-114	135-185	171-201	260-304	230-326	330-405	380-445	420-475	425-470	450-500
Observed size (bp)	X:104.65-105.45 Y:110.31-110.83	141.86-170.81	170-193.28	278.35-297.65	254.32-307.33	337.29-396.32	393.81-422.69	429.92-465.25	425.59-459.19	457.39-496.15
Out of expected range alleles (bp)	-	-	170	-	-	-	-	-	-	-
Heterozygosity (%)	-	66.3	74.7	68	85.2	94.7	88.4	81	83.1	75.7

(QF-PCR) (4). Among these methods, FISH and recently QF-PCR and MLPA showed to be more suited for high throughput screening of chromosomal numerical abnormalities (5). In order to verify the diagnostic value of QF-PCR , multiple studies have been carried in western countries (6). However, such studies are not widely available countries with high degree of consanguinity. Investigation on degree of heterozygosity in short tandem repeat (STR) markers has an importnat role in their informativity and applicability for QF-PCR in prenatal diagnosis (7,8). This study was designed to address this critical factor. Moreover, concordance between results obtained from QF-PCR and karyotyping in a double blind procedure was also assessed.

Materials and methods
Samples
Ninety-five amniotic fluid specimens were used in this study. Samples had been selected from women candidate for fetal chromosomal analysis because of increased risk in the first or second trimester screening programs. Routine chromosome analysis using standard G-banding technique was performed on all samples as conventional diagnostic test. Only Samples with excess volume were used in this study. Standard cytogenetics study including culture of amniocytes in two flasks afollowed by G-banding (at 450-band resolution) and microscopic

analysis of 20 metaphase cells was then carried out on amniotic fluid samples.

QF-PCR
DNA preparation
Three vials, each containing 1.5 ml amniotic fluid sample, were spun down at 11000 rpm for 10 minutes. The pellets of clear and blood-stained samples were washed with 200 µl 1x PBS buffer or distilled water respectively. After removing supernatant, 100 µl of InstaGene Matrix (BioRad) was added to each tube and incubated at 56ºC for 25 minutes. Tubes were then taken to 100ºC heating block for 8 minutes. After vortex and spinning down at 13000 rpm for 3 min, the tubes were placed on ice for at least 1 min before PCR.

PCR amplification
A commercial rapid aneuploidy detection kit (Chromoquant ,Sweden) was used for amplification of 20 STR markers on chromosomes 13, 18, 21, X and Y in two sets of tubes. Set1 includes primers for AMEL, D18S391, D18S976, XHPRT, D13S742, D18S386, D13S634, D13S628, D13S305 and D18S535 markers (Table 1). Second set is designed for amplification of DXS6854, DXS6803, D21S1409, SRY, X22, D21S11, D21S1246, D21S1411, D21S1444, D21S1435 markers (Table 2). PCR was carried out in 25 µl reactions containing 1.6 µl Enzyme dilution buffer and 13 µl QF-PCR buffer, both included in the kit, 0.4 µl

Table 2 : Characteristics and detected heterozygosity for multiplex 2 markers in Iranian population

	DXS6854	DXS6803	D21S1409	SRY	X22	D21S11	D21S1246	D21S1411	D21S1444	D21S1435
Location	Xq26.1	Xq21.31	21q21.2	Yp11.31	Xq28Yq	21q21.1	21q22.2	21q22.3	21q22.13	21q21.1
ExpectedSize (bp)	93-119	101-139	193-219	202-207	190-250	220-285	282-336	292-337	304-345	350-410
Observed size (bp)	91-116.10	102.81-130.88	195.64-213.77	203.73-204.11	195.14-237.04	242.13-287	293.38-327.01	290.50-336.22	304-335.42	278.87-399.44
Out of expected range alleles (bp)	91	-	-	-	-	287	-	290.50	-	-
Observed Heterozygosity (%)	68.1	80.9	74.7	-	76.8	86.3	68.4	83.1	85.2	85.2

HotStar Taq polymerase (Qiagen) and 10 µl purified DNA with final concentration of 10-20 ng per µl. PCR was programmed so that enzyme be activated at 95ºC for 15 min. Twenty-six cycles of amplification was carried out according to manufacturer's instruction.

Capillary electrophoresis

ABI 3130 Genetic Analyzer (Applied Biosystems) was used for separation of PCR products. One microliter of PCR product in conjunction with 0.3 µl of GS-500 ROX size standard (Applied Biosystems) was added to 12 µl formamide and denatured at 95ºC for 2 minutes for each lane. Tubes were placed on ice before loading. POP7 polymer (Applied Biosystems) was used for electrophoresis and results were analyzed using GeneMapper version 4 software (Applied Biosystems).

Results

As shown in Tables 1 and 2the observed sizes of alleles are narrower than expected for several markers and out of expected size ranges for others. Only one maker (D21S11) with "higher than expected" number of repeats was observed in this study. Heterozygosity percentages have been calculated for all of investigated markers. Marker D18S386 has the most heterozygosity among the markers analyzed for chromosome 18. Markers D13S634, D21S11 and X22 on chromosomes 13, 21 and X showed similar findings (Tables 1 & 2). Four fetuses affected with down syndrome were detected by both conventional and molecular cytogenetics

methods. No case affected with other chromosomal abnormalities was detected. Fifty fetuses were male and 45 were female. There was a discrepancy between results obtained by two techniques for the sex of one of the cases. QF-PCR was then repeated on cell culture and obtained results were in concordance with karyotype. It is therefore concluded that some mistake had been taken in the collection of sample for QF-PCR.

Discussion

There are only few reports of studies on STR markers in countries with high consanguinity rates (9, 10). No such studies have been carried out in West-Asian populations. Study on some STR markers, starting from their heterozygosity analysis, seems to be a fundamental stage before application of QF-PCR in prenatal diagnosis of chromosomal abnormalities. Obtained results showed that most of markers have remarkable heterozygosity and could therefore lead to high degree of informativity. These include STR markers studied on all of chromosomes 13, 18 and 21 and X. However, range of observed heterozygosity is relatively broad (Table 2). This study shows that using QF-PCR in population with high rate of consanguinity has similar potential to other countries with low rate of consanguinity. However, investigation on additional samples using more on various chromosomes are highly recommended to investigate the heterozygosity, number and distribution of common STR alleles in

order to reach a protocol with less multiplexing and more confidence in each specific population.

Acknowledgment

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