

## Investigation of QF-PCR Application for Rapid Prenatal Diagnosis of Chromosomal Aneuploidies in Iranian Population

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### Abstract

**Objective:** G-Banding followed by standard chromosome analysis is routinely used for prenatal detection of chromosomal abnormalities. In recent years, molecular cytogenetic techniques have been developed for rapid diagnosis of chromosomal abnormalities. Among these methods Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR) has been widely used for this purpose. Heterozygosity of short tandem repeat (STR) markers which leads to informativity is the most critical requirement for feasibility of QF-PCR.

**Methods:** In this study we analyzed several short tandem repeats on chromosomes 13, 18, 21, X and Y on amniotic fluid samples obtained from PND candidates to diagnose conditions such as Down, Edward and Patau syndromes and also numerical sex chromosome abnormalities such as Klinefelter and Turner syndromes.

**Findings:** Most of the analyzed STRs had acceptable heterozygosity (66.3-94.7) to be used in QF-PCR based prenatal diagnosis. Moreover, results obtained from both methods (standard karyotype and QF-PCR) for all samples were in accordance with each other.

**Conclusion:** In case of using appropriate STR markers, and in certain clinical indications, QF-PCR could be used as useful technique for prenatal diagnosis even in consanguine populations such as Iranians.

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**Key Words:** Prenatal Diagnosis; QF-PCR; Chromosomal Aberration; Short Tandem Repeats

### Introduction

Chromosomal aneuploidies, including autosomal trisomies and sex chromosome abnormalities are

among the most common causes of mental retardation (MR) and multiple congenital abnormalities in children and newborns<sup>[1]</sup>.

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Different programs have been used for prenatal screening of chromosomal abnormalities.

Invasive sampling (amniotic fluid or chorionic villi sampling) followed by standard G-Banding for high risk mothers is the current routine approach<sup>[2]</sup>. The process of sampling to final report takes at least two weeks<sup>[3]</sup>. This is a considerable problem in Iran regarding to ethical and legal concerns for termination of pregnancy after 18 weeks of gestational age.

Application of newly developed molecular cytogenetic techniques such as fluorescent in situ hybridization (FISH), multiplex ligation dependent probe amplification (MLPA) and quantitative fluorescence polymerase chain reaction (QF-PCR)<sup>[4]</sup> have recently been increased by research and diagnostic services. QF-PCR is the most recently developed method for rapid and high throughput screening of chromosomal aneuploidies<sup>[5]</sup>. Multiple pilot studies have been carried out to analyze the diagnostic power of this technique as a diagnostic method in several western populations<sup>[6]</sup>. However, there are only few reports of application of this technique in countries with high rate of consanguinity.

Heterozygosity of short tandem repeats (STRs) is proved to be a critical factor in the informativeness of these markers and for application of QF-PCR in prenatal detection (PND)<sup>[7,8]</sup>. This research was carried out to assess heterozygosity and the size range of different STR markers on chromosomes 13, 18, 21, X and Y in Iranian population.

Assessment of concordance between results obtained from QF-PCR and karyotyping in a double blind procedure was another main objective of our study.

## Subjects and Methods

### Samples:

One hundred and eighteen AF (amniotic fluid) samples were received for analysis of chromosomal aneuploidies because of increased risk in the first or second trimester screening programs. Informed consent was obtained from all families. Routine second trimester chromosome analysis using standard G-banding technique was performed on all samples as conventional

diagnostic test. Excess volume of samples was used for analysis using QF-PCR.

Amniotic cell cultures were carried out using standard cytogenetic techniques. Briefly, using G-banding technique, twenty metaphase cells were studied from cultures of amniocytes in two flasks at 450-band.

### DNA Isolation and QF-PCR:

DNA isolation and QF-PCR were carried out as previously described<sup>[9]</sup>. Briefly, using Instagene Matrix (BioRad, Cat No. 732-6030), 1 to 3 ml AF sample, was spun down at 11000 rpm for 10 minutes (Eppendorf 5415-R). The pellets of clear and blood-stained samples were washed with 200µl 1×PBS buffer or distilled water respectively. After removing supernatant, 100µl of InstaGene Matrix (BioRad) was added to each tube and incubated at 56°C for 25 minutes.

Tubes were then taken to 100°C heating block for 8 min. After vortex, spinning down at 13000 rpm for 3 min, the tubes were placed on ice for at least 1 min before PCR.

### PCR Reaction:

Chromoquant (Sweden) rapid aneuploidy detection kit was used for amplification of 20 STR markers on chromosomes 13, 18, 21, X and Y in two sets of tubes. Set 1 included primers for AMEL, D18S391, D18S976, XHPRT, D13S742, D18S386, D13S634, D13S628, D13S305 and D18S535 markers (Table 1). Second set was designed for amplification of DXS6854, DXS6803, D21S1409, SRY, X22, D21S11, D21S1246, D21S1411, D21S1444, D21S1435 markers (Table 2). PCR amplification was carried out in 25µl reactions containing 1.6µl enzyme dilution buffer and 13µl QF-PCR buffer, both included in the kit, 0.4µl HotStar Taq polymerase (Qiagen) and 10µl purified DNA with final concentration of 10-20ng per µl.

Enzymatic activation was carried out at 95°C for 15 minutes. Twenty-six cycles of amplification were carried out according to manufacturer's instruction.

### Separation of PCR products (Capillary electrophoresis):

Separation of PCR products was performed using ABI 3130 Genetic Analyzer (Applied Biosystems). For each lane, 1µl of PCR product in parallel to

**Table1:** Characteristics of "multiplex 1" markers and their heterozygosity in Iranian population

	Location	Expected Size bp	Observed size bp	Out of expected range alleles bp	Heterozygosity %
<b>AMEL</b>	Xp22.31-Xp22.1 Yp11.2	X:103-108 Y109-114	X:104.65-105.45 Y:110.31-110.83	--	--
<b>D18S391</b>	18p11.31	135-185	141.86-170.81	--	63.71
<b>D18S976</b>	18p11.31	171-201	170-193.28	170	74.77
<b>XHPRT</b>	Xq26.1	260-304	278.35-297.65	--	68
<b>D13S742</b>	13q12.13	230-326	254.32-307.33	--	84.07
<b>D18S386</b>	18q22.1	330-405	337.29-396.32	--	92.72
<b>D13S634</b>	13q21.33	380-445	393.81-422.69	--	88.07
<b>D13S628</b>	13q31.1	420-475	429.92-465.25	--	75.70
<b>D13S305</b>	13q13.3	425-470	425.59-459.19	--	78.50
<b>D18S535</b>	18q12.3	450-500	457.39-496.15	--	73.14

0.3µl of GS-500 ROX size standard (Applied Biosystems) was added to 12µl formamide and denatured at 95°C for 2 min. Tubes were then placed on ice before loading.

POP7 polymer (Applied Biosystems) was used for electrophoresis and results were analyzed using GeneMapper version 4 software (Applied Biosystems).

## Findings

Preliminary results of this study has previously been reported<sup>[9]</sup>. Results of marker heterozygosity analyses are summarized in Tables

1 and 2. Observed ranges of allele sizes are narrower than expected for markers AMEL, D18S391, XHPRT, D13S742, D18S386, D13S634, D13S628, D13S305, D18S535, DXS6803, D21S1409, X22, D21S1246, D21S1444, and D21S1435. Broader size ranges were observed for markers D18S976, DXS6854, D21S11, and D21S1411. Lower numbers of repeats were present in some patients for markers D18S976, DXS6854 and D21S141. There was only one marker, D21S11, with "higher number of repeats" than the expected range. Percentages of heterozygotes have been calculated for all of investigated markers. D18S386 marker had the highest heterozygosity among the markers analyzed for chromosome 18.

**Table2:** Characteristics of "multiplex 2" markers and their heterozygosity in Iranian population

	Location	Expected Size bp	Observed size	Out of expected range alleles	Observed Heterozygosity %
<b>DXS6854</b>	Xq26.1	93-119	91-116.10	91	68.1
<b>DXS6803</b>	Xq21.31	101-139	102.81-130.88	--	80.9
<b>D21S1409</b>	21q21.2	193-219	195.64-213.77	--	74.50
<b>SRY</b>	Yp11.31	202-207	203.73-204.11	--	--
<b>X22</b>	Xq28Yq	190-250	195.14-237.04	--	77.47
<b>D21S11</b>	21q21.1	220-285	242.13-287	287	85
<b>D21S1246</b>	21q22.2	282-336	293.38-327.01	--	68.18
<b>D21S1411</b>	21q22.3	292-337	290.50-336.22	290.50	78.78
<b>D21S1444</b>	21q22.13	304-345	304-335.42	--	84.46
<b>D21S1435</b>	21q21.1	350-410	278.87-399.44	--	80.55

**Table 3:** Results obtained from analysis of 118 at risk samples in regards of chromosomal aneuploidies

Total No	Female	Male	Down	Other Aneuploidies
118	59	59	5	-

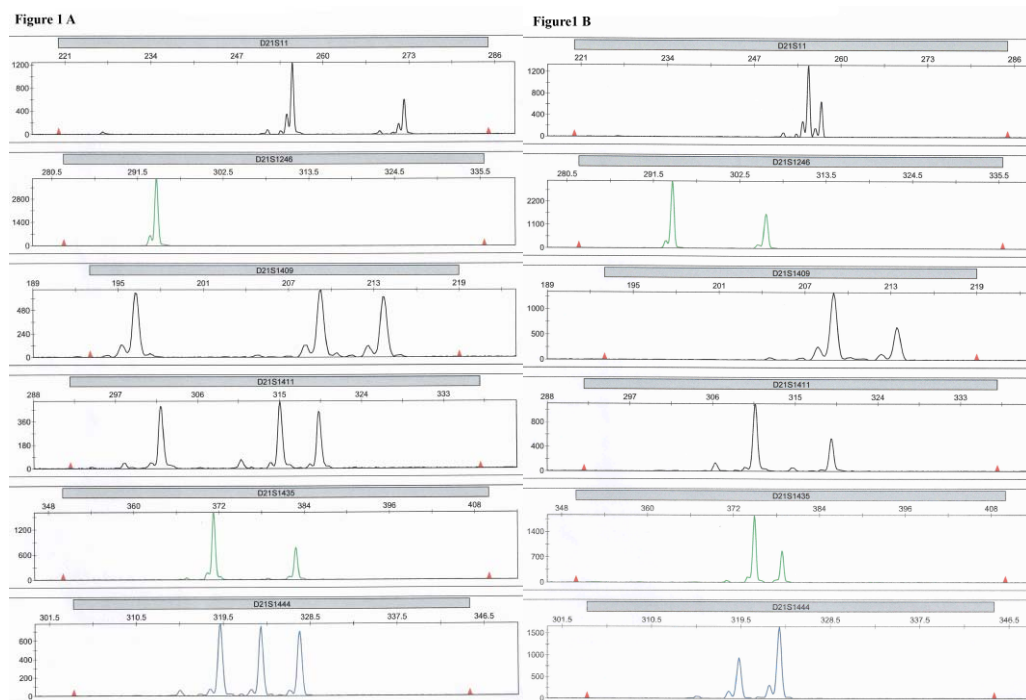
Similar condition was observed for markers D13S634, D21S11 and X22 on chromosomes 13, 21 and X respectively (Tables 1 and 2). From 118 analyzed samples 5 cases of Down syndrome were diagnosed both with conventional cytogenetics and QF-PCR (Table 3). Four out of five affected samples showed three alleles for each STR markers on different loci (Fig. 1A) but one of them had only two alleles for all loci with ratio of 1:2 (Fig. 1B). Such a profile might be seen in two conditions: isochromosome 21 or trisomy 21 with nondisjunction in meiosis II without any crossing over occurred in meiosis I. QF-PCR could not differentiate these conditions, but karyotype analyses of that sample confirmed trisomy (Fig. 2). No other chromosome abnormality was detected among the studied patients. Fifty-nine fetuses were males and 59 were females. There was a discrepancy between results obtained by two

techniques for the sex of one of the cases. QF-PCR was then performed on cultured cells and obtained results were concordant with karyotype. Sample collection error was assumed as the possible cause of this discrepancy.

## Discussion

The main aim of this study was to assess the applicability of QF-PCR for rapid prenatal detection of aneuploidy in Iranian population.

Most of reported studies about the application of QF-PCR have been performed in countries with low rate of consanguinity<sup>[9]</sup>. Furthermore, designing and validation of this method in a population needs extensive data about STR

**Fig. 1:** Plots of samples with Down syndrome with mostly triallelic and 1:1:1 ratio (A) and biallelic with 2:1 ratio (B)

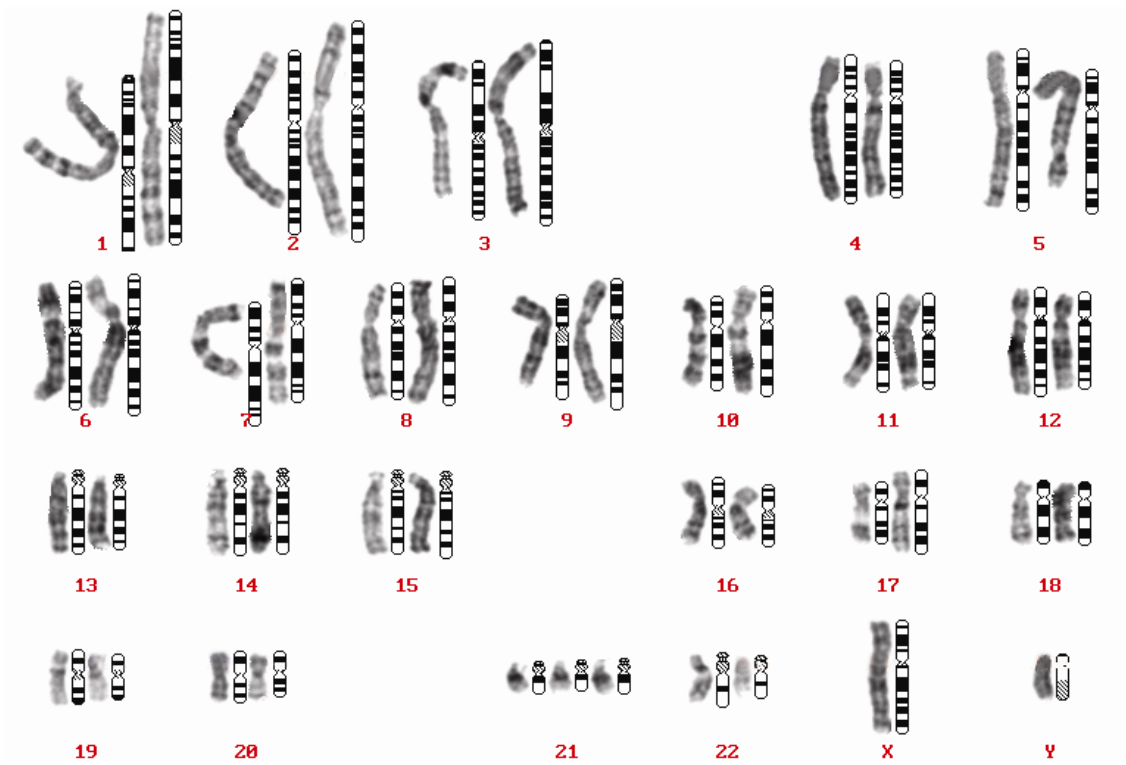


Fig. 2: A karyogram indicating a fetal Down syndrome with trisomy 21 (same sample of figure 1B).

markers such as heterozygosity and also number, distribution and size of different alleles<sup>[10]</sup>. There are not enough reports of research on STR heterozygosity in societies with high degree of consanguinity. No such studies have been reported in West-Asian countries. Analysis of some STR markers heterozygosity seems to be an essential step before utilization of QF-PCR method in prenatal diagnosis of numerical chromosome abnormalities.

In spite of our expectation aroused from high rate of consanguinity in our population, obtained data showed that most of analyzed STRs have remarkable heterozygosity and could lead to high rate of informativity in families. These results are correct for STR markers studied on all of chromosomes 13, 18, 21 and X. However, the degree of observed heterozygosity is relatively variable. For example in the case of chromosome 18 it differs from 66.3 for marker D18S391 to 94.7 for marker D18S386. Therefore we are planning to study more other markers on different chromosomes to find those with higher degree of informativity. As the time limit for legal abortion is up to 18 weeks of gestational age, there are some

ethical and legal considerations for using conventional karyotyping on amniotic fluid samples obtained after 17 weeks of gestation. Since the final results are available in less than 6 hours, this technique could therefore be used for late amniocentesis as well as other cases in decision making for abortion in legal time-limit. No false positive results were found in results obtained by QF-PCR.

## Conclusion

This study, in accordance with our preliminary reported findings and other previous studies<sup>[9-10]</sup>, provides more support for the assertion that application of QF-PCR in populations with high degree of consanguinity (like Iran) has similar applicability potential as in other societies with low degree of consanguinity.

However, more studies on various STR markers are highly recommended to analyze heterozygosity, number and distribution of

different alleles, in order to develop a specific protocol with more confidence in each population. Moreover, further studies are required to assess the validity of the technique as a standalone tool for detection of chromosomal abnormalities<sup>[12]</sup>.

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**Conflict of Interest:** None

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