TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2017) 47: 142-147
© TÜBİTAK
doi:10.3906/sag-1511-157

QF-PCR in invasive prenatal diagnosis: a single-center experience in Turkey

Özge ÖZER KAYA[1,*], Altuğ KOÇ[1], Taha Reşid ÖZDEMİR[1], Özgür KIRBIYIK[1], Berk ÖZYILMAZ[1], Mehmet ÖZEREN[2], Deniz Can ÖZTEKİN[2], Cüneyt Eftal TANER[2], Yaşar Bekir KUTBAY[1]

[1]Genetic Diagnostic Center, Tepecik Training and Research Hospital, İzmir, Turkey
[2]Department of Obstetrics and Gynecology, Tepecik Training and Research Hospital, İzmir, Turkey

Received: 25.11.2015 • **Accepted/Published Online:** 15.05.2016 • **Final Version:** 27.02.2017

Background/aim: QF-PCR has been used for more than 20 years. It is based on investigation of polymorphic short tandem repeats (STRs) and is widely used for prenatal rapid aneuploidy detection.

Materials and methods: We report retrospectively our prenatal diagnosis results between January 2012 and May 2014 in Tepecik Training and Research Hospital Genetic Diagnostic Center. Prenatal diagnosis was recommended in 6800 high-risk pregnancies and 2883 patients agreed to invasive diagnosis. Chromosome analysis and QF-PCR were performed in all patients.

Results: Normal results were reported in 2711 cases by fetal karyotyping and in 2706 cases by QF-PCR. Anomaly detection rates were similar for the two methods (5.09% for karyotyping and 4.02% for QF-PCR).

Conclusion: QF-PCR is a fast and reliable prenatal diagnosis method in all indication groups and may be preferred as the sole prenatal investigation in patients without fetal ultrasonographic findings.

Key words: Amniocentesis, chromosome aberrations, prenatal diagnosis, genetic counseling, polymorphism

1. Introduction

Several invasive techniques are used to detect fetal chromosome anomalies during the prenatal period. Until quite recently, conventional chromosome analysis of fetal samples was regarded as the gold standard for prenatal diagnosis. However, currently, for fetuses with ultrasonographic findings, cytogenetic microarray (CMA) analysis is recommended for preliminary investigations and for fetuses with other risk factors, QF-PCR analysis (1). As is the case in many other countries, conventional cytogenetic analysis is still the major diagnostic approach in this country. The main disadvantage of the conventional approach is the prolonged duration of reporting time due to long-term cell cultures. Although international guidelines suggest 2 weeks reporting time for prenatal tests (2), the actual duration of fetal karyotyping is around 3–4 weeks, especially in centers with high numbers of samples. As another disadvantage, karyotyping may give unexpected findings other than aneuploidies. The reports may increase the patient's anxiety due to uncertain clinical outcome. The diagnostic investigation may even end with unnecessary termination of the pregnancy.

Advanced maternal age and increased aneuploidy risk in maternal serum screening were reported as the major indications for prenatal cytogenetic diagnosis (3), and these indications aim to identify aneuploidies mainly. Thus, the rapid aneuploidy detection (RAD) methods are included in daily practice (4). Fluorescence in situ hybridization (FISH) was used for rapid detection initially. Direct examination of uncultured interphase cells is the major advantage of FISH but the relatively expensive and laborious procedures limit the use of rapid FISH (5).

QF-PCR has been used for more than 20 years (6). It is based on investigation of polymorphic short tandem repeats (STRs) and is used widely for prenatal rapid aneuploidy detection. Determination of trisomic aneuploidy is based on amplification of STRs. Each specific STR has a specific length according to the number of repeats, thus distinguishing one homologous chromosome from its counterpart is possible. In contrast to fetal karyotyping, QF-PCR can be carried out with very low quantities of samples in remarkably shorter periods of time. Worldwide patient series are reported for QF-PCR (1,7–9), but STR marker variations among populations lead to the necessity of population-based reports. In the present study, we report our QF-PCR experience and the informativeness of STR markers for a Turkish population.

* Correspondence: drozgeozer@gmail.com

2. Materials and methods

Samples were collected between January 2012 and May 2014 in Tepecik Training and Research Hospital Genetic Diagnostic Center. All patients were offered genetic counseling before the invasive procedure. All of the patients were informed about the procedures, limitations, possible results, and complications. Informed consent was obtained in all cases. Increased aneuploidy risks in maternal serum screening, presence of ≥2 soft markers or major malformation on fetal ultrasonography, and advanced maternal age (≥35 years at birth) were the indications for prenatal invasive diagnosis. Patients with incomplete clinical data were not included in the study.

Prenatal diagnosis was recommended for 6800 high-risk pregnancies and 2883 (42%) patients agreed to invasive diagnosis. Chromosome analysis and QF-PCR was performed for all patients. The majority (84%) of the patients (n: 2427) underwent amniocentesis (AC), 12% (n: 333) chorionic villi sampling (CVS), and 4% (n: 123) cordocentesis (CS).

Chromosome analysis was performed according to conventional methods. Short- and long-term cell cultures were used for appropriate samples; direct karyotyping was not used.

2.1. QF-PCR

Genomic DNA was isolated from a 2-mL amniotic fluid sample, 200-µL fetal blood sample, or 40–60-mg chorionic villous sample using a High Pure PCR Template Preparation Kit (Roche, USA) according to the manufacturer's instructions. Discolored amniotic fluid samples (suggested having maternal blood contamination), fetal cord blood samples, and chorionic villi samples were also compared with maternal peripheral blood samples to exclude maternal cell contamination. QF-PCR assays were performed with a commercially available Devyser Complete QF-PCR kit Version 1 (Devyser, Sweden). At least 7 STR markers for each 3 autosomal (13, 18, 21) and 2 sex (X, Y) chromosomes were analyzed, and 50-ng/µL DNA samples were used per PCR mix. PCR was performed in 25-µL total volume. PCR conditions were as follows: first denaturation at 95 °C for 15 min, 26 cycles for denaturation at 94 °C for 30 s, annealing at 58 °C for 90 s, extension at 72 °C for 90 s, and final extension at 72 °C for 30 min.

Eight microliters of PCR products were mixed with 10 µL of formamide and 0.5 µL of ROX size standard (ABI, USA) in a MicroAmp (Applied Biosystems, USA) optical 96-well reaction plate. After being denatured for 3 min at 95 °C and cooled for 3 min at –20 °C, capillary electrophoresis was performed in an ABI 3130 system (ABI, USA). The GeneScan Analysis program was used for determination of peak length and areas.

2.2. Data interpretation

The results were defined according to peak areas and described as 1:1, 1:1:1, 2:1, or uninformative (when there was only one peak). The allele dosage ratio interpretation criteria are summarized in Table 1 for informative markers. At least two informative markers were chosen to give normal results for each chromosome and at least 3 markers were needed to report an anomaly. Extra markers were used for confirmation of uninformative results. When a trisomic pattern (2:1 or 1:1:1 ratios) was detected in only one marker, parental samples were tested to exclude a partial duplication. The study was repeated at DNA isolation level in the case of amplification failure at least twice.

Heterozygosity ratios were evaluated for 22 STR markers in 605 objects that were randomly selected from the patient group. X1, X2, X3, Y1, and 7X markers are not shown in the heterozygosity results table. Patients with numerical chromosome abnormalities were excluded from the interpretation.

3. Results

The most common indication for chromosome analysis was an abnormal maternal serum screening test result (n: 1320, 45.8%). The other frequent indications were advanced maternal age (n: 888, 30.8%) and abnormal fetal ultrasound findings (n: 593, 20.6%). Relatively rare indications were maternal anxiety, Down syndrome history in early pregnancies, and familial reciprocal translocation (n: 82, 2.8%).

Chromosome analysis and QF-PCR results are shown in Table 2. Normal results were reported in 2711 cases (94.03%) by fetal karyotyping and in 2706 cases (93.86%) by QF-PCR. Anomaly detection rates were similar for the two methods (5.09% for karyotyping and 4.02% for QF-

Table 1. Allele dosage ratio interpretation criteria.

Result	Allele dosage ratio	
	Two peak distance <25 bp	Two peak distance >25 bp
Normal	<1.45	<1.52
Gray zone	1.45–1.80	1.52–1.80
Trisomy	≥1.80	≥1.80

Table 2. Genetic anomaly rates in fetal cytogenetic analysis and QF-PCR.

	Karyotype	%	QF-PCR	%
Trisomy 21	60	2.08	58	2.01
Trisomy 13	5	0.17	7	0.24
Trisomy 18	28	0.97	28	0.97
Monosomy X	13	0.45	13	0.45
Other mosaic aneuploidies*	4	0.14	0	0.00
Mosaic trisomy 13	1	0.03	0	0.00
45,X/46,XX	1	0.03	0	0.00
45,X/46,X,+mar/46,XX	1	0.03	0	0.00
Trisomy 22	1	0.03	0	0.00
Triploidy	3	0.10	3	0.10
XX/XY mosaicism	2	0.07	0	0.00
XXX	2	0.07	2	0.07
XXY	5	0.17	5	0.17
Balanced rearrangement	16	0.55	0	0.00
Unbalanced rearrangement	5	0.17	1	0.03
Culture or amplification failure	25	0.87	4	0.14
Uninformative results for one or more chromosome	-	-	20	0.69
Maternal cell contamination	0	0.00	37	1.28
Normal	2711	94.03	2706	93.86
TOTAL ANOMALY	147	5.10	116	4.02

* Mosaic aneuploidies other than 13, 18, 21, X, and Y.

PCR). No false positive results were observed for either method.

Maternal cell contamination (MCC) was detected by QF-PCR in 37 cases and the detection rate was highest in the CVS group (11 of 333 cases, 8%). The material type and result comparison is shown in Table 3 for the MCC group.

QF-PCR failed due to detection of only one informative marker, maternal cell contamination, or amplification failure in 61 cases (2.11%) (Table 2). In total, 20 samples had just one informative marker for sex chromosomes (n: 9, one of them was mosaic 45,X), chromosome 21 (n: 6), chromosome 18 (n: 2), chromosome 13 (n: 2), and for both chromosomes 18 and 21 (n: 1). Culture failure was seen in 0.91% of fetal karyotyping studies.

Discordant results among QF-PCR and fetal karyotyping are summarized in Tables 4 and 5.

Among the 111 cases with nonmosaic numerical chromosomal abnormalities detected by fetal karyotyping, three of them were not detected by QF-PCR. The detection failure in this group by QF-PCR was due to amplification failure and maternal contamination for two of the cases. For one of them, the case was monosomy X could not be analyzed because of an inadequate number of informative sex chromosome makers.

Heterozygosity ratios are shown in Table 6 with STR locus for markers. Polymorphic aberrations (2:1 or 1:1:1 ratio for one marker) were observed in D21S11, D13S634, D13S742, D13S628, D21S1412, and D21S1446 markers; almost all of them were inherited parentally and seemed to be benign.

Table 3. Comparison of QF-PCR results between different sample types MCC group.

	AC	CVS	CS
45,X/46,X,+mar/46,XX	1	-	-
46,XX/46,XY	1	-	1
Normal	20	7	-
Trisomy 21	1	-	-
Culture failure	1	4	1

AC: Amniocentesis, CVS: Chorion villus sample, CS: Cordocentesis.

Table 4. Discordance results and testing indications.

	Abnormal maternal serum screening test results	AMA	Abnormal fetal ultrasound findings	Family history
Balanced (apparently) rearrangement	8	3	2	3
Unbalanced rearrangement	2	1	1	0
Unbalanced numerical abnormality	1	3	3	0

AMA: Advanced maternal age.

Table 5. Number of abnormalities detected by one method but not the other.

Chromosomal abnormality	Number of patients (%)	Failed method	Comment
Trisomy 21	2 (0.07)	QF-PCR	One amplification failure and one MCC
Trisomy 13	2 (0.07)	Chromosome analysis	Culture insufficiency
Other mosaic aneuploidies*	4 (0.14)	QF-PCR	No marker for related regions
Mosaic trisomy 13	1 (0.03)	QF-PCR	Possible low level mosaicism
45,X/46,XX	1 (0.03)	QF-PCR	MCC
45,X/46,X,+mar/46,XX	1 (0.03)	QF-PCR	Uninformative markers for sex chromosomes
Trisomy 22	1(0.03)	QF-PCR	No marker for related regions
XX/XY mosaicism	2 (0.07)	QF-PCR	MCC
Balanced rearrangement	16 (0.55)	QF-PCR	Out of detection capability
Unbalanced rearrangement	4 (0.14)	QF-PCR	No marker for related regions

4. Discussion

QF-PCR is a routine diagnostic tool for screening frequent chromosomal aneuploidies. Previously, researchers have suggested that it is a fast, cheap, and reliable diagnostic method (10–12). In our population, QF-PCR is widely used but no large series were reported to date (13).

In our daily routine, sole advanced maternal age is still a frequent indication of prenatal diagnosis. In previous reports, the main indication for prenatal diagnosis was advanced maternal age (≥35 years); in contrast to those reports we showed that increased aneuploidy risk in maternal serum screening is the major invasive prenatal test indication (3). Efficient use of first trimester maternal serum screening combined with fetal nuchal translucency measurement is the reason for the distinct indication frequencies among previous and recent studies.

The aneuploidy detection capability of QF-PCR is determined according to 3 factors: test failure, false positive, and false negative rates (14). QF-PCR failure was reported as 1.3% due to maternal cell contamination and the failure ratio for conventional cell culture (karyotyping) was 0.12%–0.3% (14). In our study the MCC rate was similar: 1.28%. Most of the MCC was detected in the CVS group. Seven patients with MCC were detected by QF-PCR, although they were reported as normal by fetal karyotyping. Therefore, genetic counseling about MCC rates is obviously needed for fetal karyotyping by CVS.

In our study, just 4 cases (0.14%) could not be reported by QF-PCR due to amplification failure. In contrast, 25 (0.87%) cases were not reported by fetal karyotyping due to culture failure. QF-PCR has lower failure rates.

Previously, in the literature, it was underlined that the main factor for evaluation of QF-PCR performance was number of STR markers (14). In our study, 7 markers are used for each chromosome (13, 18, 21, X, Y). Compatible with previous reports (7,10,13), we had no false positive results.

Mosaic cases are important for the management of pregnancy in clinical practice. Low level mosaicism may not be detected by molecular methods. In our study mosaic trisomy 13, 45,X/46,XX, and 45,X/46,X,+mar/46,XX karyotypes were detected by chromosome analysis in 3 cases but not by QF-PCR. Analysis reports should be prepared carefully for such situations and pretest genetic counseling should include mosaicism risk.

QF-PCR is used as a stand-alone test for selected indications in United Kingdom (15). It has been accepted that merely QF-PCR could be an efficient method for screening chromosome aneuploidies for referrals without fetal ultrasound findings. Distinct opinions about this issue

Table 6. Heterozygosity ratios of STR markers used in QF-PCR study.

Marker ID	STR LOCUS	Informative	Uninformative	Heterozygosity (%)
13A	D13S742	556	48	92.05
13B	D13S634	526	76	87.38
13C	D13S628	440	156	73.83
13D	D13S305	497	101	83.11
13E	D13S800	443	157	73.83
13F	D13S252	465	136	77.37
13G	D13S325	460	142	76.41
18A	D18S391	388	215	64.34
18B	D18S978	439	164	72.8
18C	D18S535	476	129	78.68
18D	D18S386	552	48	92
18G	D18S976	455	147	75.58
18J	D18S976	453	145	75.75
18M	GATA178F11	508	95	84.25
21A	D21S1435	468	128	78.52
21B	D21S11	492	104	82.55
21C	D21S1411	528	76	87.42
21D	D21S1444	459	139	76.76
21E	D21S2039	450	134	77.05
21F	D21S1412	501	100	83.36
21G	D21S1446	448	146	75.42
XY2	DXYS267	475	126	79.03

are present (16). In our study, just one case seems to be missed if we used QF-PCR solely in fetuses without ultrasound findings. Therefore, we suggest that it is convenient to use QF-PCR as a stand-alone test in this group.

Polymorphic STR duplications have been discussed before and assessment of parental samples has been suggested to exclude partial trisomies (7). In our study, a polymorphic trisomic pattern was observed in 24 cases (data not shown). All of these results were confirmed with parental studies and accepted as normal variant or polymorphic changes. Commercially available STR markers or QF-PCR kits are used for routine testing. Nevertheless these polymorphic markers could be specific for each population. Therefore, we present heterozygosity ratios for our population. These findings will be helpful for future studies or diagnostic applications in Turkish populations.

From an ethical perspective, it has been speculated that patients should choose their prenatal diagnosis method (17). As an alternative approach, we suggest that QF-PCR may be recommended to all indication groups at first, and patients with normal QF-PCR results and fetal anomaly may undergo CMA analysis as the second step of the prenatal investigation.

The presented report is an example of a routine prenatal diagnostic work-up in Turkey. In conclusion, it is obvious to regard QF-PCR as a fast and reliable prenatal diagnosis method in all indication groups and it may be used as the sole prenatal investigation in patients without fetal ultrasonographic findings.

References

1. Caine A, Maltby AE, Parkin CA, Waters JJ, Crolla JA; UK Association of Clinical Cytogeneticists (ACC). Prenatal detection of Down's syndrome by rapid aneuploidy testing for chromosomes 13, 18, and 21 by FISH or PCR without a full karyotype: a cytogenetic risk assessment. Lancet 2005; 366: 123-128.
2. Prenatal Diagnosis Best Practice Guidelines (2009) v1.00. http://www.acgs.uk.com/media/765666/acc_prenatal_bp_dec2009_1.00.pdf. Accessed September 28, 2015.

3. Cogulu O, Ozkinay F, Akin H, Onay H, Karaca E, Durmaz AA, Durmaz B, Aykut A, Pariltay E, Kirbiyik O et al. Reasons for adult referrals for genetic counseling at a genetics center in Izmir, Turkey: analysis of 8965 cases over an eleven-year period. J Genet Couns 2011; 20: 287-293.

4. Hixson L, Goel S, Schuber P, Faltas V, Lee J, Narayakkadan A, Leung H, Osborne J. An overview on prenatal screening for chromosomal aberrations. J Lab Autom 2015; 20: 562-573.

5. Hultén MA, Dhanjal S, Pertl B. Rapid and simple prenatal diagnosis of common chromosome disorders: advantages and disadvantages of the molecular methods FISH and QF-PCR. Reproduction 2003; 126: 279-297.

6. von Eggeling F, Freytag M, Fahsold R, Horsthemke B, Claussen U. Rapid detection of trisomy 21 by quantitative PCR. Hum Genet 1993; 91: 567-570.

7. Cirigliano V, Voglino G, Ordoñez E, Marongiu A, Paz Cañadas M, Ejarque M, Rueda L, Lloveras E, Fuster C, Adinolfi M. Rapid prenatal diagnosis of common chromosome aneuploidies by QF-PCR, results of 9 years of clinical experience. Prenat Diagn 2009; 29: 40-49.

8. Nicolini U, Lalatta F, Natacci F, Curcio C, Bui TH. The introduction of QF-PCR in prenatal diagnosis of fetal aneuploidies: time for reconsideration. Hum Reprod Update 2004; 10: 541-548.

9. Mann K, Hills A, Donaghue C, Thomas H, Ogilvie CM. Quantitative fluorescence PCR analysis of >40,000 prenatal samples for the rapid diagnosis of trisomies 13,18 and 21 and monosomy X. Prenat Diagn 2012; 32: 1197-1204.

10. Putzova M, Soldatova I, Pecnova L, Dvorakova L, Jencikova N, Goetz P, Stejskal D. QF-PCR-based prenatal detection of common aneuploidies in the Czech population: five years of experience. Eur J Med Genet 2008; 51: 209-218.

11. Lau ET, Tang L, Wong C, Yung Hang L, Ghosh A, Leung WC, Sin WK, Lau TK, Kung YY, Tang MH. Assessing discrepant findings between QF-PCR on uncultured prenatal samples and karyotyping on long-term culture. Prenat Diagn 2009; 29: 151-155.

12. Baig S, Ho SS, Ng BL, Chiu L, Koay ES, Leow GH, Gole L, Kothandaraman N, Chan J, Sun X et al. Development of quantitative-fluorescence polymerase chain reaction for the rapid prenatal diagnosis of common chromosomal aneuploidies in 1,000 samples in Singapore. Singapore Med J 2010; 51: 343-348.

13. Onay H, Ugurlu T, Aykut A, Pehlivan S, Inal M, Tinar S, Ozkinay C, Ozkinay F. Rapid prenatal diagnosis of common aneuploidies in amniotic fluid using quantitative fluorescent polymerase chain reaction. Gynecol Obstet Invest 2008; 66: 104-110.

14. Langlois S, Duncan A. Use of a DNA method, QF-PCR, in the prenatal diagnosis of fetal aneuploidies. J Obstet Gynaecol Can 2011; 33: 955-960.

15. Hills A, Donaghue C, Waters J, Waters K, Sullivan C, Kulkarni A, Docherty Z, Mann K, Ogilvie CM. QF-PCR as a stand-alone test for prenatal samples: the first 2 years' experience in the London region. Prenat Diagn 2010; 30: 509-517.

16. Lildballe DL, Vogel I, Petersen OB, Vestergaard EM. Diagnostic performance of quantitative fluorescence PCR analysis in high-risk pregnancies after combined first-trimester screening. Dan Med J 2014; 61: A4964.

17. de Jong A, Dondorp WJ, Timmermans DR, van Lith JM, de Wert GM. Rapid aneuploidy detection or karyotyping? Ethical reflection. Eur J Hum Genet 2011; 19: 1020-1025.