

Validation of QF-PCR for Rapid Prenatal Diagnosis of Common Chromosomal Aneuploidies in Korea

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Purpose: Quantitative fluorescent polymerase chain reaction (QF-PCR) allows for the rapid prenatal diagnosis of common aneuploidies. The main advantages of this assay are its low cost, speed, and automation, allowing for large-scale application. However, despite these advantages, it is not a routine method for prenatal aneuploidy screening in Korea. Our objective in the present study was to validate the performance of QF-PCR using short tandem repeat (STR) markers in a Korean population as a means for rapid prenatal diagnosis.

Material and Methods: A QF-PCR assay using an Elucigene kit (Gen-Probe, Abingdon, UK), containing 20 STR markers located on chromosomes 13, 18, 21, X and Y, was performed on 847 amniotic fluid (AF) samples for prenatal aneuploidy screening referred for prenatal aneuploidy screening from 2007 to 2009. The results were then compared to those obtained using conventional cytogenetic analysis. To evaluate the informativity of STR markers, the heterozygosity index of each marker was determined in all the samples.

Results: Three autosomes (13, 18, and 21) and X and Y chromosome aneuploidies were detected in 19 cases (2.2%, 19/847) after QF-PCR analysis of the 847 AF samples. Their results are identical to those of conventional cytogenetic analysis, with 100% positive predictive value. However, after cytogenetic analysis, 7 cases (0.8%, 7/847) were found to have 5 balanced and 2 unbalanced chromosomal abnormalities that were not detected by QF-PCR. The STR markers had a slightly low heterozygosity index (average: 0.76) compared to those reported in Caucasians (average: 0.80). Submicroscopic duplication of D13S634 marker, which might be a unique finding in Koreans, was detected in 1.4% (12/847) of the samples in the present study.

Conclusion: A QF-PCR assay for prenatal aneuploidy screening was validated in our institution and proved to be efficient and reliable. However, we suggest that each laboratory must perform an independent validation test for each STR marker in order to develop interpretation guidelines of the results and must integrate QF-PCR into the routine cytogenetic laboratory workflow.

Key Words: Prenatal diagnosis, Quantitative fluorescent polymerase chain reaction (QF-PCR), Short tandem repeats (STR), Aneuploidy

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Introduction

Rapid prenatal detection of numerical chromosome abnormalities by quantitative fluorescent polymerase chain reaction (QF-PCR) allows for reliable prenatal diagnosis of trisomies 13, 18, and 21^{1, 2)}. QF-PCR is an alternative method for rapid aneuploidy detection (RAD) of common aneuploidies based on the amplification of chromosome-specific DNA short-tandem-repeat (STR) polymorphisms, offering an attractive alternative to fluorescent in situ hybridization (FISH). Efforts to compare FISH and QF-PCR have concluded that both perform well in terms of overall sensitivity and specificity^{3, 4)}. However, the FISH method is a labor-intensive procedure, and also requires quite a large amount of AF to perform both FISH and culture-based karyotyping.

Large-scale studies using QF-PCR for rapid prenatal diagnosis of major chromosome aneuploidies demonstrated that the method is highly efficient, reliable, and cost effective^{5, 6)}. The QF-PCR technique allows for detection of common aneuploidies—usually within 24–48h—and provides rapid reassurance for all women with normal results. This approach requires accurate, robust, and rapid assays with a well-considered selection of STR markers because the allele frequency and heterozygosity rates (or informativity) of STR markers vary among different populations⁷⁾. Here, we report the QF-PCR results of 847 AF samples for trisomies 13, 18, 21, and X and Y from a three year period (2007–2009) at our institution and have evaluated the performance of QF-PCR in a Korean population.

Materials and Methods

A total of 847 AF samples that were referred to our institution for prenatal aneuploidy screening from 2007 to 2009 were used in the present study. Both QF-PCR and conventional cytogenetics studies were performed on all samples. Informed consent for genetic testing

was obtained from all subjects.

DNA was extracted from 1 to 2 mL of AF using a QIAamp DNA Mini Kit (QIAGEN, GmbH, Germany) according to the manufacturer's instructions, and 1.25–10 ng of DNA was used for PCR. A QF-PCR assay was performed using an Elucigene QST*R plus kit (Gen-Probe, Abingdon, UK) in which 20 STR markers were included: 5 markers for each chromosome such as 13, 18, 21, X and Y. The amplified DNA samples were analyzed with ABI 3130xl and Genotyper 3.7 (Applied Biosystems, Foster City, USA). Peak height ratios between 0.8 and 1.4 were defined as normal; three equal peak heights or peak height ratios between 1.8 and 2.4 suggested trisomy. Single peak or allele ratios between 1.4 and 1.8 were interpreted as uninformative markers, and the final conclusion required more than one informative marker. Samples with less than two informative markers on each chromosome were re-tested using chromosome-specific QF-PCR, including two STRs on chromosome 21, four STRs on chromosome 18, three STRs on chromosome 13, and seven STRs on the sex chromosomes. All samples were processed and reported within 24h.

To evaluate the informativity of the STR markers, the heterozygosity of each marker was determined in all 847 samples and compared with that of Caucasian (CEPH database, <http://www.cephb.fr>) and Southeast Asian populations⁸⁾.

Conventional cytogenetic analyses were performed according to standard procedures⁹⁾. The results of QF-PCR were compared to those obtained by conventional cytogenetic analysis from cultured cells.

Results

Eight hundred and forty-seven AF samples were tested using QF-PCR. The major indications for QF-PCR were an abnormal maternal serum screening test results (82.5%), followed by advanced maternal age (5.3%), and abnormal ultrasound findings (4.0%). The

results of the QF-PCR and conventional cytogenetic analyses are given in Table 1. Out of the 828 cases diagnosed as normal by QF-PCR, 821 cases were diagnosed as normal by conventional cytogenetics tests. Chromosomal aneuploidies were detected in 19 cases (2.2%, 19/847) after QF-PCR analysis of the 847 AF samples (Table 1). They included 11 cases diagnosed as trisomies 21 (Fig. 1A), 18 (Fig. 1B), and 13. Their results are identical to those of conventional cytogenetic analysis, with 100% positive predictive value. The QF-PCR method was also highly successful in the detection of fetuses with triploidies (69,XXX) (Fig. 1C) and sex chromosome abnormalities (Turner syndrome, 47,XXY, and 47,XYY). However, after cytogenetic analysis, 7 samples (0.8%, 7/847) were found to have 5 balanced structural abnormalities [46,XY,inv(5)(p15.3q11.2), 46,XX,inv(3)(p25q25.3), 2 cases of 45,XY,der(13;14)(q10;q10) and 46,XX,t(1;3)(q21.3;p21.3)] and 2 unbalanced structural abnormalities [46,XY,dup(7)(p15.3p22) and 45,X,add(15)(p11.2)], which could not be detected by QF-PCR.

The detection of heterozygous patterns with fluorescent peak ratios close to 1:1 for at least two chro-

sosome-specific STRs was sufficient to perform the diagnosis. There was no failure to amplify the STR markers. The majority of normal samples showed diallelic peaks with a ratio of 1:1 for each STR marker. Maternal cell contaminations were detected in 12 (0.4%) samples. Owing to extra alleles or skewed ratios between peaks for all chromosomes (Fig. 1D), the results from these samples were not interpreted and the sample was reported unsuitable. Cytogenetic analysis of cultured cells from these samples showed a normal karyotype.

The STR markers for the three autosomes had a low heterozygosity value (average: 0.76), except for certain markers (D13S305, D13S800, D18S386, and D21S1435), compared to the value reported in Caucasians (average: 0.8) and had more similarity to the value reported in Southeast Asians (average: 0.78) (Table 2). The observed allele number of each STR marker was from 9 to 36 (Table 2). Twelve cases (1.4%) showed D13S634 submicroscopic duplication patterns, which were in 1:1:1 or 2:1 ratios, with the normal pattern of other STR markers for chromosome 13 and a normal karyotype (Fig. 1E).

Table 1. Results for the 847 AF Samples by QF-PCR and Cytogenetic Analysis

Karyotype	QF-PCR	Cytogenetics
46,XX or 46,XY	828	821
47,XX,+21 or 47,XY,+21	8	8
47,XX,+18 or 47,XY,+18	2	2
47,XX,+13 or 47,XY,+13	1	1
45,X	3	3
47,XXX	1	1
47,XXY	1	1
47,XYY	2	2
69,XXX	1	1
Balanced structural rearrangements*	not detected	5
Unbalanced structural rearrangements**	not detected	2
Maternal cell contaminations†	12	0
Total abnormalities	19	26

*46,XY,inv(5)(p15.3q11.2), 46,XX,inv(3)(p25q25.3), 2 cases of 45,XY,der(13;14)(q10;q10), 46,XX,t(1;3)(q21.3;p21.3)

**46,XY,dup(7)(p15.3p22), 45,X,add(15)(p11.2)

†QF-PCR results showing evidence of maternal cell contamination have been considered unsuitable for diagnosis

Discussion

QF-PCR was first introduced in 1993¹⁰. Over the years, this technique has been developed and made available for rapid prenatal diagnosis of common aneuploidies^{11, 12}. Before the routine use of QF-PCR, FISH was the only molecular diagnostic technique for rapid prenatal diagnosis of aneuploidies, and, in many countries, has commonly been performed for more than 10 years¹³. Recently, some other molecular techniques, such as multiple ligation-dependent probe amplification (MLPA), have been introduced for the rapid screening of aneuploidies¹⁴. Individually, these methods have a number of advantages and disadvantages. The main advantages of QF-PCR are: (1) the small amount of AF required; (2) there is no need to culture fetal cells; and

Table 2. Observed Alleles and Heterozygosity Index of STR Markers for Three Autosomes (13, 18, and 21) and the Reported Heterozygosity Index of other Populations

Chromosome	Marker	Observed alleles no.	Heterozygosity index		
			Korean (present study)	Caucasians (CEPHDB)	Southeast Asian population (Quaife et al.,2004)
13	D13S252	9	0.74	0.88	–
	D13S305	20	0.88	0.86	0.8
	D13S628	11	0.74	0.69	0.65
	D13S634	19	0.88	0.81	0.84
	D13S800	9	0.81	0.74	–
18	D18S386	36	0.91	0.82	0.85
	D18S390	12	0.6	0.75	–
	D18S535	11	0.81	0.93	0.76
	D18S819	10	0.57	0.75	–
	D18S978	10	0.89	0.93	–
21	D21S11	17	0.78	0.78	0.81
	D21S1409	10	0.61	0.81	–
	D21S1435	10	0.79	0.7	–
	D21S1437	13	0.7	0.78	–
	D21S1446	13	0.67	0.78	–
Average heterozygosity index			0.76	0.8	0.78

(3) the speed with which the test can be automated, allowing for a high sample throughput. Compared to other rapid prenatal diagnosis techniques (e.g., FISH and MLPA), detection of maternal cell contamination is one of the most important features of QF-PCR^{15, 16)}. The maternal and fetal XX cells are indistinguishable by FISH, rendering maternal cell contamination undetectable from female fetuses.

In the present study, the overall results showed that QF-PCR is a rapid, simple, and accurate diagnostic test. In 821 cytogenetically normal pregnancies, which were correctly diagnosed by QF-PCR—without false positive results—parents could be informed of the outcome of the test within 24h from the collection of the sample. The greatest positive attribute of QF-PCR is its potential to reduce parental anxiety. As shown in Table 1, QF-PCR performed on AF samples correctly diagnosed 100% of trisomies for chromosome 13, 18, and 21, triploidies, and non-mosaic aneuploidies involving both chromosomes X and Y without false negative results. No false positive results were observed (100% positive predictive value). These results are in agreement with the published data showing the QF-PCR

method to be accurate in a clinical setting^{12, 17, 18)}. In some laboratories, sex chromosome-specific QF-PCR are not routinely performed for all cases¹²⁾; only samples retrieved from fetuses suspected by ultrasound results of having such chromosome disorders are tested¹⁸⁾. However, sex chromosome aneuploidies detected in the present study such as three Turner syndromes, one 47,XXY, one 47,XXX, and two 47,XYY cases, are generally not referred as a result of an abnormal ultrasound. We strongly suggest that STR markers for the QF-PCR diagnosis of sex chromosome aneuploidies should routinely be included; early detection of all such cases would leave more time for the parents to receive the appropriate genetic counseling^{20–23)}.

A total of 19 aneuploidies have been readily detected by QF-PCR in the present study; this accounts for 73.1% (19/26) of fetuses with abnormal karyotypes diagnosed by conventional cytogenetic tests (Table 1). Although QF-PCR and FISH are reliable for the detection of common aneuploidies, reports indicate that 15–30% of chromosome abnormalities detected by karyotyping would not be detected by QF-PCR or FISH^{3, 24, 25)}. Seven cases (0.8%, 7/847) not detected by QF-PCR

included the five balanced structural rearrangements, which have an associated risk of the existence of physical or developmental effects if they arise de novo, whereas inherited rearrangements, unknown before prenatal testing. In case of application of QF-PCR as a stand-alone test, careful genetic counseling is essential to explain the limitations of QF-PCR, including the inability to detect all chromosomal abnormalities, as well as the possibility of uninformative or false-negative results in some cases.

In the present study, the QF-PCR results could not be reported for 1.4% (12 cases) of samples showing evidence of maternal cell contamination. Most of these

specimens were blood-stained AFs. QF-PCR amplification of highly polymorphic STRs of a sample heavily contaminated by maternal cells is expected to produce a characteristic pattern with extra alleles or skewed ratios between peaks for all chromosomes (Fig. 1D). These patterns are not usually compatible with a normal, trisomic, or triploid result, so these samples can be safely tested without any risk of misdiagnosis. In the presence of low-level maternal cell contamination, the ratio between fetal STR peaks is not significantly altered; in these cases, diagnosis can be performed without great difficulty and in the presence of female fetuses by testing maternal DNA with the same markers.

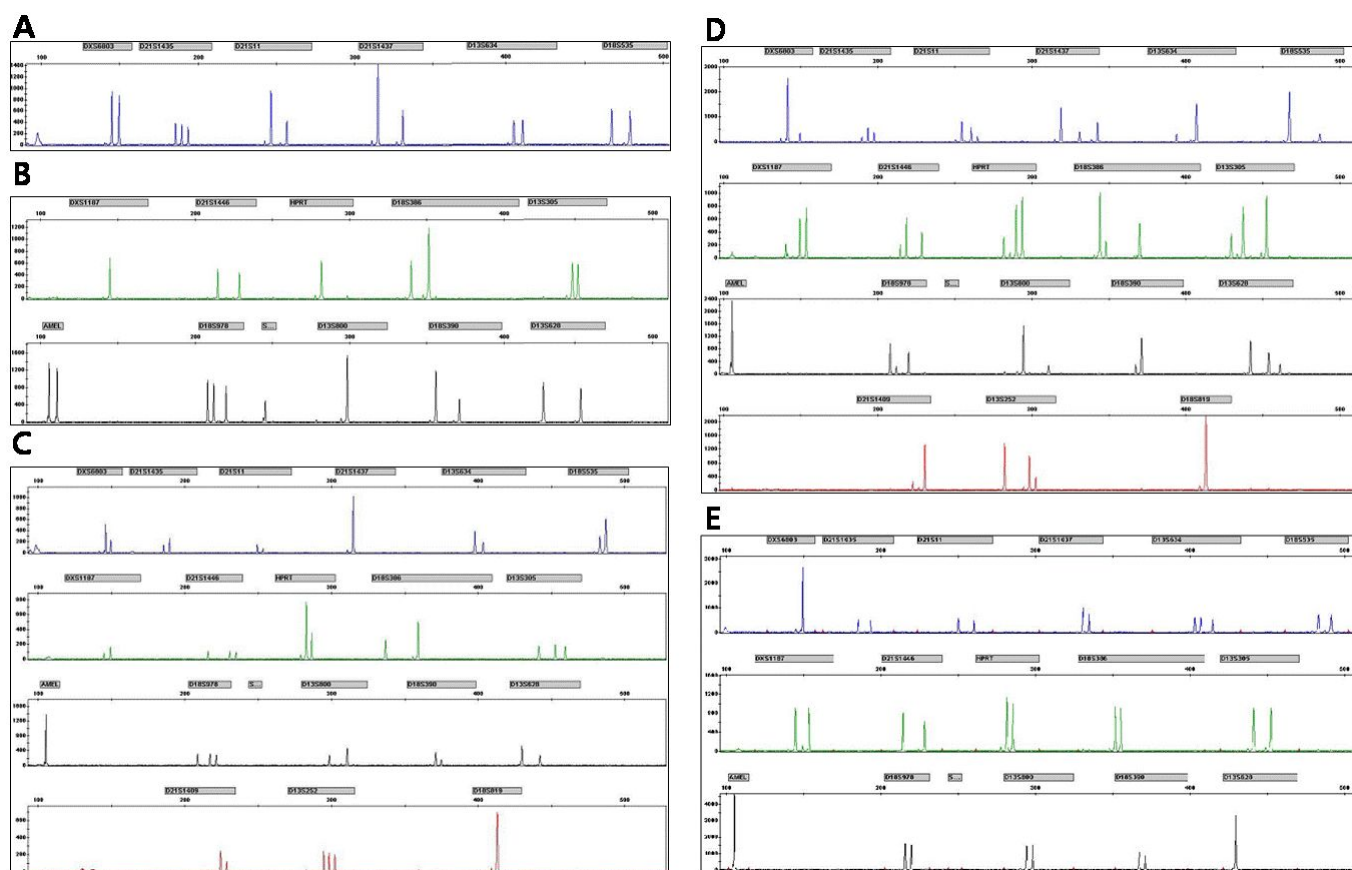


Fig. 1. Electrophoretograms of the AF samples amplified with QF-PCR and analyzed with ABI 3130xl and Genotyper 3.7. The x-axis shows the length of the PCR products in base pairs and the y-axis shows the fluorescence intensity in arbitrary units. (A) trisomy 21 case with the triallelic pattern of a 1:1:1 ratio (D21S1435) and diallelic pattern of a 2:1 ratio (D21S11 and D21S1437) in chromosome 21 STR markers; (B) trisomy 18 case with the triallelic pattern of a 1:1:1 ratio (D18S978) and diallelic pattern of a 2:1 ratio (D18S386 and D18S390) in chromosome 18 STR markers; (C) triploidies (69,XXX) showing pattern of trisomy at all informative STR markers on all chromosomes; (D) maternal cell contamination showing a characteristic pattern with extra alleles or skewed ratios between peaks for all chromosomes; (E) submicroscopic duplications in D13S634 with the triallelic pattern of a 1:1:1 ratio and normal diallelic pattern of other STR markers.

Laboratories performing QF-PCR in prenatal diagnosis usually use their own individual marker combinations or a commercially available kit including specific STR markers for chromosomes such as 13, 18, 21, X and Y²⁶⁾. In the present study, the STR markers for three autosomes (13, 18, and 21) had a lower heterozygosity index than those of Caucasians, except for D13S305, D13S800, D18S386, and D21S1435. In spite of a lower heterozygosity index in the Korean population, all samples, with the exception of two cases, showed more than one informative markers of each chromosome. The excluded two cases were required analysis with additional markers. The average heterozygosity index (0.76) of the STR makers found in this study is slightly higher than that detected in previous report for Korean populations (0.74)²⁷⁾.

Submicroscopic duplications of the microsatellite were observed in 12 cases (1.4%) as clear triallelic or diallelic patterns for one chromosome-specific STR marker, D13S634 (Fig. 1E). They all had a normal karyotype. We could not obtain parental blood samples, which would have helped in determining the inheritance patterns and the clinical significance of the submicroscopic duplication of D13S634. This finding was detected in the previous report for the Korean population by Cho et al.²⁷⁾ with the similar frequency (1.6%). A relatively high proportion of submicroscopic duplication patterns in D13S634, which is located in 13q21.33, might be a unique finding in the Korean population when compared with the data reported in Western countries²¹⁾. The STR submicroscopic duplication and somatic microsatellite mutation observed in the D21S11 marker in another Korean report, by Lee et al.⁶⁾, was not detected in the present study.

In conclusion, QF-PCR for prenatal aneuploidy screening was validated in our institution and proved to be efficient and reliable. However, we suggest that each laboratory that uses this method perform an independent validation test in order to develop the interpretation guidelines of results, and each laboratory must

also integrate QF-PCR into the normal cytogenetics laboratory workflow.

국문초록

목적: QF-PCR법은 혼란 염색체 이수성에 대한 빠른 산전 진단을 가능하게 하는데, 낮은 가격, 빠른 속도, 그리고 자동화가 가능하여 한꺼번에 많은 검체에 대해 적용할 수 있다는 장점이 있다. 하지만 아직까지 국내에서 QF-PCR법은 산전 염색체 이수성 선별검사로 주로 사용되는 방법이 아니다. 본 연구에서는 한국인에서 빠른 산전 진단을 목적으로 시행하는 짧은 염기서열 반복(short tandem repeats, STR) 표지자를 이용한 QF-PCR법의 수행능을 검증하고자 한다.

대상 및 방법: 2007년에서 2009년까지 산전 염색체 이수성 선별을 목적으로 의뢰된 847개의 양수 검체에 대해 QF-PCR법을 시행하였는데 13번, 18번, 21번, X, Y염색체에 위치한 총 20개의 STR 표지자로 구성된 Elucigene kit (Gen-Probe, Abingdon, UK)를 사용하였다. 총 847개의 양수 검체에 대한 QF-PCR 결과는 염색체 검사 결과와 비교하였고, STR 표지자의 정보력을 평가하기 위해서 각 표지자에 대해 이형접합체 지수(heterozygosity index)를 구하였다.

결과: 총 847개 양수 검체에 대한 QF-PCR 검사 결과 19개(2.2%, 19/847)에서 13, 18, 21번 염색체와 X, Y염색체의 수적 이상이 관찰되었는데 염색체 검사에서도 동일한 결과를 보여 100% 양성 예측율을 나타냈다. 하지만 염색체 검사 결과 7개(0.8%, 7/847) 검체에서 5개의 균형전좌와 2개의 불균형 염색체 이상이 관찰되었으나 QF-PCR에서는 진단되지 않았다. STR 표지자의 평균 이형접합체 지수(heterozygosity index)는 0.76으로 서양인에서 보고된 0.8에 비해 다소 낮았다. 본 연구에서 D13S634 표지자의 미세수준의 중복(submicroscopic duplication)이 1.4% (12/847)에서 관찰되었는데 이는 한국인에서 특징적인 소견으로 생각된다.

결론: 본 기관에서는 산전 염색체 이수성 선별을 위한 QF-PCR법을 검증하였으며 효율적이고 신뢰할 수 있는 방법임이 입증되었다. 하지만 QF-PCR 결과를 해석하기 위한 지침을 만들기 위해서 검사실마다 독립적으로 각각의 STR 표지자에 대한 검증이 필요하며, 또한 QF-PCR법을 통상적인 염색체 검사 업무흐름에 통합하는 것이 필요하다고 사료된다.

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