Performance of MLPA as a screening method for aneuploidy in uncultured amniocytes

Anöploidilerin kültür edilmemiş amniyositlerde multiplex ligation dependent amplification (MLPA) yöntemi ile saptanması

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Abstract

Objective: To test whether the Multiplex Ligation-dependent Probe Amplification (MLPA) technique can be used as a screening test for rapid diagnosis of aneuploidies in uncultured amniocentesis.

Material and Methods: In this prospective blind study, MLPA with chromosomes 13,18,21,X and Y specific probe mixes was performed in 500 amniotic fluid samples. Chromosome copy numbers were determined by analyzing size and peak area for each MLPA probe. Results were compared with those of karyotyping/FISH.

Results: Conclusive test results were obtained in 98% of the samples, whereas 10 were inconclusive. In all conclusive tests, the MLPA results were concordant with that of cytogenetic and/or FISH analyses. There were no false-positive results. A case with 69,XXX triploidy could not be diagnosed by MLPA. In total, 28 aneuploidies were diagnosed. There were no false-positive results. The performance of each probe was determined.

Conclusion: MLPA is a rapid, simple and reliable assay for aneuploidy screening in uncultured amniocytes.

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Key words: MLPA, prenatal screening, common aneuploidies, uncultured amniocytes

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Özet

Amaç: MLPA tekniği ile prenatal dönem anöploidi tanısı yeni, alternatif bir metoddur. Çalışmamızda bu yeni tekniğin prenatal tanı testi olarak rutinde kullanılabilirliğinin sınanması, tekniğin sensivite, spesivite ve test başarısızlık oranlarının saptanması ve bu yeni yöntemin rutinde kullanılan diğer anöploidi tanı yöntemlerine göre avantaj ve dezavantajlarının belirlenmesi amaçlanmıştır.

Gereç ve Yöntemler: Toplam 500 hastanın amniyon sıvısında MLPA tekniği ile 13., 18., 21., X ve Y kromozomları için doz tayini yapılmıştır. MLPA tekniği ile saptanan sonuçlar, bu hastalara ait diğer rutin yöntemlerle saptanan sonuçlar ile karşılaştırılmıştır.

Bulgular: Tekniğin anöploidi tanısınındaki sensivitesi %100, spesivitesi %100 ve test başarısızlık oranı %4 olarak saptanmıştır. 69,XXX karyotipli örneğimizde MLPA tekniği ile doğru sonuç alınamamıştır.

Sonuç: MLPA te kniği ile prenatal tanıda anöpoidi tayininin pratik, hızlı ve güvenilir şekilde yapılabileceği düşünülmüştür.

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Anahtar kelimeler: MLPA, prenatal tanı, anöploidi, kültüre edilmemiş amniyositler

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Introduction

Prenatal diagnosis for genetic disorders was first carried out in the 1970s, and since then the most common indication for prenatal diagnosis remains an increased risk of having a child with trisomy syndromes of chromosomes 21,13,18 and sex chromosome aneuploidies. They account for 60-80% of abnormal fetal karyotypes detected in amniotic fluid cells (1). Fetal karyotyping has been the gold standard for diagnostic testing for over 30 years and no new technology could be proven usable for the detection of numerical and/or structural abnormalities for all chromosomes. However, a number of molecular methods based on uncultured fetal cells have been developed to reduce the waiting time related with prenatal chromosome analysis. Molecular methods including FISH and multiplex quantitative fluorescence-PCR (QF-PCR) are now in common practice for the rapid prenatal diagnosis of the most prevalent chromosome abnormalities (2, 3). These technologies importantly decrease the reporting times from 2-3 weeks to 1-2 days (4-6). Although they are informative for only commonly seen aneuploidies, such a rapid result is important in cases of abnormal US findings or for obstetric managements, and is valuable for relieving parental stress during the necessary culture period.

Recently, a new technique, Multiplex Ligation dependent Probe Amplification (MLPA), has emerged for the relative quantification of about 40 different DNA sequences in a single reaction. It was first described in 2002 and it has been shown to have many potential applications in diagnostic cytogenetic and molecular genetics (7). A MLPA kit for rapid aneuploidy detection is commercially available. The experiences in prenatal samples using MLPA is promising, but further studies

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need to be reported to know the limitations and performance of the MLPA tests. In this prospective blind study, we present the results of 500 consecutive amniocentesis samples analyzed by computer assisted MLPA analysis. The sensitivity and specifity percentages of the technique and each of the probes were addressed in the study.

Materials and Methods

In this prospective blind study, a total of 500 amniotic samples were referred to the cytogenetics section of the Department for karyotyping. Referral reasons covered all the prenatal diagnosis indications including maternal age (≥35), increased Down Syndrome risk based on maternal serum screening and/or nuchal thickness measurement, ultrasound detected abnormalities or anxiety. The first 2ml of amniotic fluid drawn was discarded because of maternal cell contamination. Usually, 15-20 ml of amniotic fluid samples were obtained and 2 ml was taken for MLPA testing. Blood contaminated samples were excluded. Of all samples, 450 (89%) were between 15 and 18 weeks whereas 80 were between 24 and 30 weeks.
G-banding analysis and direct-FISH analysis by using AneuVision Probe Set (Vysis) were carried out by using standard techniques. Since the aim of this study was to test MLPA (SALSA MLPA kit P095 Aneuploidy Lot 0307, 1206, 1106, 0505) analysis prospectively, the MLPA data were interpreted without knowing FISH and/or karyotyping results.

Sample preperation and analysis

DNA from 2 μl amniotic fluid was isolated by using QIAamp kit (Qiagen) according to the manufacturer's instructions. In total, 45-150 ng DNA was used in the MLPA protocol. The MLPA assay was performed according to the manufacturer's protocol with small modifications. Briefly, 5μl of lysate were denaturated for 5 min at 98^0C, and then 3 μl probe mix were added and the mix was heated at 950C for 1 min and incubated at 60^0C overnight (16 hours). By using mineral oil, the problems arising from evaporation were solved.
The ligation was performed at 540C for 15 min by adding 32μl heat-stable ligase-65 enzyme into the hybridization product. The reaction mix including the 10μl ligation mix was preheated at 950C for one minute, followed by 35 cycles (30 sec at 950C, 30 sec at 60^0C and 60 sec at 72^0C). A measure of 2 μl PCR product was analyzed by capillary electrophoresis on an ABI Prism 310 Genetic Analyzer with Rox-500 size standards. DNA samples from three males and three females were spontaneously used as external normal controls.

MLPA data analysis

By visual analysis of peak profiles, test results were defined as conclusive if MLPA quality control fragments showing sufficient genomic DNA was present in the mixture. Genescan 3.7 and genotyper 3.6 software were used in the analysis of size and peak area for each MLPA probe and the data were exported to a Microsoft Excel based Coffalyser v1.4 program. For quantification purposes, the relative peak area for each probe was calculated as a fraction of the total sum of peak areas in a given sample. Each autosomal peak fraction was divided by the median peak fractions of that locus for all samples in that reaction.
The relative probe signal values between 0.7 and 1.3 were defined as normal. If the value of target sequence was lower than 0.7, it was defined as monosomy, whereas trisomy was defined if the value is ≥1.3.

Results

The MLPA analyses were performed in 500 amniotic fluid samples. Conclusive test results were obtained in 98% of the samples, whereas 10 samples were "inconclusive". The internal MLPA quality control fragments indicated an insufficient amount of genomic DNA in these 10 samples. However, no correlation was determined between the failure of MLPA analysis and gestational age at sampling. In all conclusive tests, the MLPA test for chromosomes 13, 18 and 21 was concordant with that of cytogenetic and/or FISH analyses. The criterion in the trisomy diagnosis was that at least four of eight chromosome-specific probes should have a relative probe signal higher than 1.3. By using this criterion, autosomal trisomy diagnosis was revealed in 24 samples (trisomy 21: 18 samples, trisomy 18: 4 samples and trisomy 13: 2 samples).
In the fetal sex determination, the presence of relative probe signals for X chromosome and Y chromosome specific probes were diagnosed as male, whereas the samples without Y chromosome specific signal but with X chromosome specific probe signals were diagnosed as female. All fetal sex results were consistent with the karyotyped sexes. In two cases, although there were no Y chromosome specific signals, relative probe signals specific to X chromosome were <1.3 in the range from 0.910 to 1.230. These two cases were diagnosed as monosomy X and the results were confirmed by the cytogenetic analysis. In one case, not only were there higher X chromosome specific signals in the ranges from 1.350 to 2.00, but also all Y chromosome specific signals (1.08-1.100) were seen and therefore the gonosomal chromosome constitution of the case was diagnosed as XXY. The result was in accordance with the fetal karyotype (47, XXY). The diagnosis of the other case was XXX since the relative signal ratio of all eight X chromosome specific probes were ≥ 2.0 (ranges in between 2.00 and 3.170).
Although the sex chromosome constitution of the case was revealed as XX by the MLPA, the karyotype of the fetus was 69, XXX triploidy. The triploidy could not be diagnosed by the MLPA. Neither autosomal nor sex chromosomes specific probes showed higher relative probe signals. As seen in "Table 1", the higher (≥1.3) relative probe signal ratio was only seen in the ABCC4 probe specific to chromosome 13, but no higher ratio was determined in the other 23 autosomal specific probe signals.
In the evaluation of the performance of the MLPA in aneuploidy screening, the sensitivity and specifity of the test were determined as 97% and 100%, respectively on the basis of 490 samples with conclusive results. As seen in "Table 2", in a trisomy diagnosis of either chromosome 13, 18 or 21, almost all chromosome-specific probes have a relative probe signal >1.3. Besides, in the diagnosis of sex chromosome aneuploidies, all eight X chromosome-specific probes and four Y chromosome specific probes allowed a correct diagnosis "Table 3". The sensitivity and false-positive rates for all autosomal trisomy probes were determined on the basis of the 490 samples with conclusive results , "Table 4". While only 8 of 24 autosomal specific

Table 1. Relative probe signals in a case with 69,XXX karyotype

Probe Name	Chromosome	Length PCR	Ratio
SIM2	21q22.2	136	0.840
MADH4	18q21.1	142	0.910
ABCC4	13q32	148	1.390
AR	Xq11.2	154	1.480
SRY	Yp11.3	160	0.000
NCAM2	21q21.1	166	1.14
PMAIP1	18q21	172	1.05
CCNA1	13q12.3	178	0.700
FACL4	Xq23	184	1.660
SRY	Yp11.3	193	0.000
USP25	21q11.2	202	1.080
SS18	18q11.2	211	0.730
RB1	13q14.3	220	0.890
ARX	Xp22.1	229	1.410
UTY	Yq11	238	0.000
STCH	21q11	247	0.870
NFATC1	18q23	256	0.940
DACH	13q21.3	265	1.06
TM4SF2	Xp11.4	274	1.550
ZFY	Yp11.3	283	0.00
SOD1	21q22.1	292	0.950
TYMS	18p11.3	301	0.890
P85SPR	13q34	310	1.010
L1CAM	Xq28	319	1.600
APP	21q21.3	337	1.260
SERPINB2	18q21.3	346	1.12
BRCA2	13q12.3	355	1.16
RPS6KA3	Xp22.2	364	1.210
TFF1	21q22.3	382	1.110
SS18	18q11.2	391	0.780
DLEU1	13q14.3	400	0.82
PDCD8	Xq25	409	1.600
TIAM1	21q22.1	427	1.000
MC2R	18p11.2	436	0.95
ING1	13q34	445	1.210
DMD	Xp21.2	454	1.530

probes have a sensitivity lower than 100%, the false-positive rate for all probes is below 0.1%. The sensitivity percentage of two chromosome 13 specific probes (85SPR and BRCA2) was 50% but their specifity was100%. The highest false-positive rate was seen in ING1 chromosome 13 specific probe, the others were below 0.1%. The sensitivity rates of X chromosome-specific probes were 100%. However, the false-positive percentages of two X chromosome specific probes (AR and L1CAM) were higher compared to that of autosomal probes “Table 4”.

Since it is a screening method for the detection of copy number alterations of chromosomes 13, 18, 21, X and Y, the structural aberrations of these chromosomes and aneuploidies other than these chromosomes could not be detected by this approach. In the clinical series of this study, a fetus with a balanced t(21;21) translocation and two fetuses with structural chromosome aberrations could be diagnosed by karyotyping.

Discussion

MLPA is a rapid technique for prenatal aneuploidy detection in a routine diagnostic laboratory. This is the first study in Turkey related to the data of MLPA used in a clinical series of 500 amniocentesis samples. Although the samples obtained from the 15th week to 30th week of gestation were analyzed, conclusive results were obtained in 98% of the samples and therefore the results showed that the MLPA test is usable until late pregnancy. Inconclusive results were obtained in 10 samples because of insufficient amount of DNA. However, no correlation was seen between the failure of MLPA tests and gestational age at sampling, but the cell content of the sample was an important factor for a reliable MLPA test. In six of these samples, the cell content of the fluid was significantly lower and there were some difficulties in karyotyping these samples, as well. The MLPA probe mix used in this study included four DQ (DNA Quantity) control fragments. These short fragments (in range 64-82 nt long) are very informative since they give off a clear warning signal if the amount of sample DNA is lower than the amount of DNA required for a reliable MLPA test (8). The amplification products of the DQ fragments are only visible when little or no DNA is present, and even when the ligation did not occur. Ten samples with inconclusive results in the present study showed the amplified DQ fragments. Our experiences showed that 2ml of amniotic fluid is sufficient to perform a conclusive test, but the cell content of the sample is becoming an important factor in the reliability of the test.

In the present study, the MLPA-diagnosed trisomic fetuses and fetuses with X chromosomal aneuploidies were confirmed by the cytogenetic and/or direct FISH analyses. However, a fetus with triploidy could not be diagnosed by MLPA but was determined by direct-FISH analysis in a 24h duration test. The inability to detectpolyploidy is one of the main limitations of the MLPA assay and this has also been reported previously (9-13).

If the sensitivity and specifity of the probes were evaluated individually, our experiences showed that most of the probes had 100% sensitivity. The lowest sensitivity rate was 50% seen in the P85SPR probe specific to chromosome 13 “Table 4”. The probes TYMS and SS18 specific to chromosome 18 had 75% sensitivity. However, the specificity of these probes was 100% on the basis of 490 samples with conclusive result. The false-positive rate of all probes was below 0.1%, but X chromosomal probes AR and L1CAM had higher false-positive rates than the other X-chromosome and autosome specific probes “Table 4”. The high false-positive rate for X chromosomal specific probe

Table 2. Relative probe signals in fetuses with trisomy 13, 18 and 21

Tri-13 Probes	1234	1350	Tri-18 probes	1506	1631	1041	1640											
ABCC4	1.620	1.360	MADH4	1.670	1.790	1.370	1.600											
CCNA1	1.640	1.720	PMAIP1	1.50	1.53	1.47	1.38											
RB1	1.640	1.480	SS18	1.40	1.35	1.410	1.360											
DACH	1.36	1.39	NFATC1	1.310	1.350	1.410	1.480											
P 85SPR	1.460	1.290	TYMS	1.05	1.360	1.460	1.360											
BRCA2	1.48	1.284	SERPINB2	1.32	1.43	1.36	1.40											
DLEU1	1.40	1.39	SS18	1.400	1.190	1.310	1.600											
ING1	1.780	1.640	MCZR	1.36	1.31	1.43	1.38											
Tri-21 Probes	**803**	**811**	**1128**	**1415**	**1868**	**1591**	**1770**	**1864**	**1499**	**1948**	**1954**	**1014**	**1316**	**1358**	**1367**	**1527**	**1670**	**1888**
SIM2	1.57	1.420	1.380	1.800	1.340	1.400	1.350	1.340	1.340	1.380	1.40	1.50	1.40	1.42	1.56	1.50	1.40	1.36
NCAM2	1.74	1.47	1.37	1.70	1.92	1.52	1.40	1.42	1.92	1.37	1.82	1.64	1.33	1.59	1.54	1.39	1.46	1.32
4SP25	1.590	1.460	1.360	1.60	1.60	1.360	1.460	1.850	1.600	1.360	1.480	1.430	1.310	1.390	1.340	1.480	1.340	1.310
STCH	1.600	1.290	1.400	1.830	1.32	1.580	1.390	1.420	1.370	1.090	1.370	1.310	1.360	1.420	1.480	1.210	1.340	1.320
SOO1	1.610	1.320	1.420	1.420	1.35	1.500	1.50	1.200	1.560	1.520	1.560	1.830	1.340	1.400	1.340	1.440	1.340	1.460
APP	1.700	1.430	1.330	1.530	1.430	1.350	1.530	1.42	1.340	1.300	1.340	1.620	1.340	1.600	1.260	1.360	1.400	1.380
TFF1	1.870	1.320	1.520	1.420	1.510	1.390	1.450	1.38	1.800	1.420	1.800	1.410	1.480	1.450	1.380	1.40	1.480	1.380
TIAM1	1.450	1.500	1.450	1.450	1.330	1.500	1.530	1.42	1.380	1.420	1.380	1.380	1.390	1.190	1.450	1.520	1.500	1.420

Table 3. Relative probe signals in fetuses with sex chromosomal aneuploidies

		Monosomy X		XXY	XXX
Probes	**Chromosome**	**1788**	**1953**	**1778**	**1003**
AR	Xq11.2	1.030	0.980	1.770	2.140
SRY	Yp11.3	0.000	0.000	1.040	0.000
FACL4	Xq23	1.080	1.027	1.490	3.170
SRY	Yp11.3	0.000	0.000	1.100	0.000
ARX	Xp22.1	1.010	1.070	1.430	3.050
UTY	Yq11	0.000	0.000	1.06	0.000
TM4SF2	Xp11.4	1.230	0.890	2.000	2.570
ZFY	Yp11.3	0.000	0.000	1.08	0.000
L1CAM	Xq28	1.000	1.030	1.760	2.000
RPS6KA3	Xp22.2	0.880	1.040	1.350	2.890
PDCD8	Xq25	0.930	1.000	1.970	2.840
DMD	Xp21.2	0.910	0.920	1.350	2.040

AR has also been reported previously (10). These unexpected false-positive data might be due to recently detected large-scale copy-number variations (LCV) or copy-number polymorphisms (CNP) spanning from several kilobases to megabase pairs of DNA (14-17). However, population–specific variations might also be involved in these false-positive results. Mutations or polymorphisms very close to the probe ligation site may cause a reduced peak area. In MLPA, amplification of probes by PCR depends on the presence of small specific target sequences in the sample. Nucleotide mismatches at the probe binding site prevent probe hybridization and ligation and therefore single base changes may result in deletions (7, 18). Therefore, in these variations, the relative signal ratios of the other probes specific to the related chromosome should be analyzed in detail.

The widely diverging sensitivity of the MLPA probes in aneuploidy screening has been discussed in previous studies (7, 10, 19). Slater et al. (19) reported false-negative results but they did not document the probes, whereas the false-positive rate in the study of Hochstenbach et al. (10) was between 0.0 % and 4.2% and they reported that only a few probes have 100% sensitivity. The differences might also be due to the differences in probe mixtures, since the SALSA P095 probe mix used in this study is an improved version of the old SALSA MLPA P001 probe mix and it has been mentioned in the MRC-Holland page (8) that the new version is less sensitive to variations in the quality of DNA.

In conclusion, the high sensitivity and specifity rates and low failure rate showed that the MLPA assay can be used as a rapid aneuploidy screening test in uncultured amniocytes. The test is inexpensive and the result can be revealed in 2-3 days,,which is very helpful for parental anxiety. However, the inability of the test to detect structural chromosome abnormalities, chromosome aneuploidies other than common chromosome syndromes and the mosaic status of fetus must always be taken into consideration. Because of these limitations, we suggested that the MLPA assay can be performed in clinical diagnostic laboratories together with fetal karyotyping.

Conflict of interest

No conflict of interest is declared by authors.

Table 4. Performance of probes in detection of autosomal and sex chromosomal aneuploidies

Tri-13 Probes	Sensitivity	False-positive	Tri-18 probes	Sensitivity	False-positive	Tri-21 probes	Sensitivity	False-positive
ABCC4	100	0.00	MADH4	100	0.04	SIM2	100	0.01
CCNA1	100	0.07	PMAIP1	100	0.00	NCAM2	100	0.00
RB1	100	0.00	SS18	100	0.08	4SP25	100	0.02
DACH	100	0.1	NFATC1	100	0.02	STCH	85.7	0.06
P85SPR	50	0.00	TYMS	75	0.09	SOO1	95.2	0.00
BRCA2	50	0.00	SERPINB2	100	0.00	APP	95.2	0.08
DLEU1	100	0.00	SS18	75	0.00	TFF1	100	0.00
ING1	100	0.14	MCZR	100	0.00	TIAM1	95.2	0.09
X chromosome Specific Probes	**45,X Sensitivity**	**False-positive**	**XXY Sensitivity**	**False-positive**	**XXX Sensitivity**	**False-positive**		
AR	100	0.00	100	0.02	100	0.5		
FACL4	100	0.08	100	0.00	100	0.00		
ARX	100	0.03	100	0.00	100	0.02		
TM4SF2	100	0.00	100	0.00	100	0.03		
L1CAM	100	0.00	100	0.00	100	0.4		
RPS6KA3	100	0.02	100	0.00	100	0.00		
PDCD8	100	0.00	100	0.00	100	0.00		
DMD	100	0.00	100	0.09	100	0.02		

References

1. R.L. Nussbaum, R.R. McInnes and H.F. Willard : Prenatal Diagnosis. In: R.L. Nussbaum, R.R. McInnes and H.F. Willard, Editors, Thompson and Thompson genetics in medicine (6th ed.), W.B. Saunders, Philadelphia, PA (2001).
2. Spathas DH, Divane A, Maniatis GM, Ferguson-Smith ME, Ferguson-Smith MA. Prenatal detection of trisomy 21 in uncultured amniocytes by fluorescence in situ hybridization: a prospective study. Prenat Diagn 1994; 14: 1049-54.
3. Mansfield ES. Diagnosis of Down syndrome and other aneuploidies using quantitative polymerase chain reaction and small tandem repeat polymorphisms. Hum Mol Genet 1993; 2: 43-50.
4. Cirigliano V, Lewin P, Szpiro-Tapies S, Fuster C, Adinolfi M. Assessment of new markers for the rapid detection of aneuploidies by quantitative fluorescent PCR (QF-PCR).Ann Hum Genet 2001; 65: 421-7.
5. Cirigliano V, Voglino G, Marongiu A, Cañadas P, Ordoñez E, Lloveras E et al. Rapid prenatal diagnosis by QF-PCR: evaluation of 30,000 consecutive clinical samples and future applications.Ann N Y Acad Sci 2006; 1075: 288-98.
6. 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: 279-97.
7. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 2002; 30: e57.
8. MRC-Holland page: http://www.mrc-holland.com/pages/indexpag.html
9. Gerdes T, Kirchhoff M, Lind AM, Larsen GV, Schwartz M, Lundsteen C. Computer-assisted prenatal aneuploidy screening for chromosome 13, 18, 21, X and Y based on multiplex ligation-dependent probe amplification (MLPA).Eur J Hum Genet 2005; 13: 171-5.
10. Hochstenbach R, Meijer J, van de Brug J, Vossebeld-Hoff I, Jansen R, van der Luijt RB, et al. Rapid detection of chromosomal aneuploidies in uncultured amniocytes by multiplex ligation-dependent probe amplification (MLPA). Prenat Diagn 2005; 25: 1032-9.
11. Bruno DL, Burgess T, Ren H, Nouri S, Pertile MD, Francis DI, et al. High-throughput analysis of chromosome abnormality in spontaneous miscarriage using an MLPA subtelomere assay with an ancillary FISH test for polyploidy Am J Med Genet A. 2006; 140: 2786-93.
12. Diego-Alvarez D, Rodriguez de Alba M, Cardero-Merlo R, Diaz-Recasens J, Ayuso C, Ramos C et al. MLPA as a screening method of aneuploidy and unbalanced chromosomal rearrangements in spontaneous miscarriages. Prenat Diagn 2007; 27: 765-71.
13. Shaffer LG, Bui TH. Molecular cytogenetic and rapid aneuploidy detection methods in prenatal diagnosis. : Am J Med Genet C Semin Med Genet 2007; 145: 87-98.
14. Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y et al. Detection of large-scale variation in the human genome. Nat Genet 2004; 36: 949–51.
15. Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P et al. Large-scale copy number polymorphism in the human genome. Science 2004; 305: 525–8.
16. de Vries BB, Pfundt R, Leisink M, Koolen DA, Vissers LE, Janssen IM et al. Diagnostic genome profiling in mental retardation. Am J Hum Genet 2005; 77: 606-16.
17. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD et al. Global variation in copy number in the human genome. Nature 2006; 444: 444-54.
18. Sellner LN, Taylor GR. MLPA and MAPH: new techniques for detection of gene deletions. Hum Mutat 2004; 23: 413-9.
19. Slater HR, Bruno DL, Ren H, Pertile M, Schouten JP, Choo KH. Rapid high throughput prenatal detection of aneuploidy using a novel quantitative method (MLPA). J Med Genet 2003; 40: 907-12.