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APPLICATION OF MLPA (MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION) IN FETUSES WITH AN ABNORMAL SONOGRAM AND NORMAL KARYOTYPE

NORMAL KARYOTİPLİ PATOLOJİK ULTRASON BULGUSU OLAN FETUSLARDA MLPA (MULTİPLEX LİGATİON-DEPENDENT PROBE AMPLİFİCATİON) UYGULAMALARI

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ABSTRACT

Objective/Material and Method: Cryptic chromosomal imbalances contribute significantly to the etiology of multiple congenital anomalies with or without mental retardation (MCA/MR). Current approaches in prenatal diagnosis include targeted high resolution analyses by MLPA and some microarray platforms or a genomewide screening at maximal resolution using oligonucleotide or SNP arrays. The major disadvantages of the latter approach are cost and the inadvertent detection of copy number variation of unknown clinical significance.

In this prospective work, fetal DNA samples from 66 fetuses who had pathological antenatal ultrasonography findings with normal karyotype and Multiprobe T-FISH results were tested using commercially available targeted MLPA probe-sets to compare the efficacy and the impact of MLPA testing at prenatal setting.

Results: Three submicroscopic deletions (3.66; 4.5%) were detected in the cohort. Two of them were de novo deletions, 18ptel and 7q11.23. The third finding was a 75 kb duplication at 18q, which was maternally inherited and probably a benign copy number variation unrelated to the pathological ultarsonography findings.

Conclusion: The observed detection rate by MLPA testing can be considered within the expected range. Furthermore, benign copy number variation was identified with the targeted diagnostic approach as an unexpected finding. This study shows that MLPA is a practical and cost-effective technique to investigate submicroscobic chromosomal aberrations in fetuses.

Keywords: MLPA, subtelomeric anomalies, prenatal diagnosis microdeletion/microduplication

ÖZET

Amaç/Gereç/Yöntem: Multiple konjenital anomaliler ve bilişsel yetersizliklerin (MKA/MR) etiyolojisinde kriptik kromozom düzensizlikler önemli yer tutmaktadır. Yeni teknolojilerin gelişmesi, MLPA veya genom boyu SNP ve oligonükleotidlerle tarama yapabilen mikrodizin ya da yeni nesil dizileme teknolojileri yüksek düzeyde çözünürlükle bu anomalilerin prenatal dönemde saptanabilmesine olanak sağlamıştır. Yüksek çözünürlüklü çalışmaların en büyük dezavantajı maliyet ile beklenmeyen ve/veya klinik önemi bilinmeyen kopya sayısı varyantlarının saptanmasıdır.

Bu prospektif çalışmada patolojik ultrason bulgusu saptanan ve normal karyotipe sahip çoklu telomerik FISH ile normal sonuç alınmış 66 fetusa ait DNA örnekleri ticari olarak satılan MLPA prob setleri ile değerlendirilerek MLPA nın tanı akış şemasındaki etkinliği araştırıldı.

Bulgular: Çalışmada toplam üç olguda (3:66; 4,5%) delesyon belirlendi. İki olguda submikroskobik de novo delesyon saptandı ve bunlardan biri 18ptel ve diğeri 7q11.23 delesyonu idi. Bir diğer olguda klinik bulgularla ilişkisiz, yüksek olasılıkla zararsız 75 kb büyüklüğünde anneden kalıtılan 18q duplikasyonu belirlendi.

Sonuç: Patojenik mutasyon saptama oranı beklenti ile uyumlu idi. Ek olarak, hedefe yönelik tanıda sıra dışı kabul edilen bir durum olarak bir olguda maternal kalıtımlı yüksek olasılıkla zararsız bir kopya sayısı değişikliği saptandı. Bu çalışma prenatal uygulamalarda submikroskobik kromozomal anomalilerin araştırılmasında MLPA tekniğinin uygun maliyetli ve kullanılabilir olduğunu göstermiştir.

Anahtar Kelimeler: MLPA, subtelomerik değişimler, doğum öncesi tanı mikrodelesyon/mikroduplikasyon

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INTRODUCTION

Chromosomal imbalances are important in the etiology of congenital malformations of the newborn period (1, 2). When major malformations are detected by fetal ultrasonography (USG), the rate of chromosomal aberrations can be as high as 29% depending on the week of pregnancy, tissue type or technique applied (3-7). Conventional karyotyping allows the genome-wide detection of chromosome anomalies at a rather low resolution (>8-10 Mb) depending on the banding level. In the presence of distinct phenotypes in postnatal cases, the fluorescence in situ hybridisation (FISH) technique using syndrome specific probes may be applied to diagnose known deletion/duplications smaller than 6Mb. Unbalanced rearrangements in gene-rich subtelomeric regions, have been identified as a significant etiological contributor to MCA/MR (8-12). Due to the nonspecific banding pattern, even larger unbalanced cryptic subtelomeric rearrangements can be easily missed by conventional karyotyping, especially when the banding level is lower than 500 bands per haploid set. MLPA and array-CGH techniques/microarray developed during the last decade have been very effective at overcoming those limitations (12-17). The rate of clinically relevant copy number changes in prenatal cases with MCA after a normal result in conventional karyotyping was reported to be 4% by FISH [12] and 5%-10 % by a-CGH technique (12, 15). While microarrays became the first-tier test in postnatal cytogenetic diagnosis (15, 16), their application in the prenatal setting are still met with reservations, mainly due to the concomitant diagnosis of variants of unknown significance (VOUS) - especially in high resolution arrays. MLPA with probe sets covering known disease associated critical regions may offer an acceptable practical compromise, trading off cost-effectiveness, sensitivity and the risk of encountering VOUS (14, 16, 17, 18-26).

Sixty-six DNA samples from fetuses with pathological USG findings and normal karyotype and subtelomeric FISH results were tested using MLPA probe sets SALSA P070 and P245 to identify the efficacy and the impact of MLPA testing at prenatal setting.

MATERIAL AND METHODS

Sixty-six archived DNA samples consecutively collected between November 2007 and April 2010 met the inclusion criteria of pathological USG findings (at least one major anomaly diagnosed by an experinced perinatologist), as well as a normal karyotype (500 bands at minimum) and normal Multiprobe T-FISH (Cytocell) analysis. Five cases with congenital heart defects along with normal TUPLE1 (Cytocell) test results and one case with lissencephaly and normal LIS1, FLI1 (Cytocell) test results were included in the study. The DNA had been extracted from fresh materials and/or cell cultures and kept frozen at -200C using the QIAamp DNA Blood Mini Kit (Qiagen, CA, USA) in 58 samples (25 fetal blood, 23 amniotic fluid and 10 chorionic villi samples) and by an automatic nucleic acid purification system (Magna Pure Compact – Roche) in 8 samples (fetal blood). All cases were tested with both SALSA P070 specific for subtelomeres and SALSA P245 specific for known microdeletion syndromes. Coffalyser v9.4 software was used for data analysis. Anomalies detected by MLPA were confirmed by FISH using commercially available probes (Cytocell and Vysis) or array-CGH (Affymetrix or Nimblegene platforms).

All the clinical examinations on the fetuses and newborns and the genetic testing were performed at the Department of Medical Genetics, Istanbul Medical Faculty of Istanbul University. Fetal USG and invasive procedures were performed at the Perinatology Division of the Obstetrics and Gynecology Department of the same faculty. Families were informed about the study and written informed consent was obtained.

RESULTS

The mean maternal age was 28.22 years (range 19-43) and the mean gestational age was 23. 8 weeks (range 16-35). Fetal sex distribution was 1:1 (33 males/33 females).

In addition to the abnormal USG findings 11 mothers out of 66 were 35 years or older, in five pregnancies maternal serum screening showed an increased risk and two mothers had a history of recurrent fetal losses.

The MLPA study revealed chromosomal imbalances in three cases (3:66; 4.5%). Two anomalies were de novo and detected with SALSA MLPA P070 probe mix and one familial anomaly with SALSA MLPA P245 probe mix.

Case-1 (MLPA#10)

The mother aged 35 was referred due to antenatal ultrasonographic finding of IUGR and diaphragmatic hernia, and an increased risk for trisomy 18 in the first trimester screening test (>1:50). Conventional karyotyping and subtelomeric FISH following amniocentesis at 17 weeks gestation revealed normal results.

The MLPA SALSA P070 probe mix showed a gain at 18q23 (Figure 1a). Although FISH analysis using 18q subtelomeric probe (Cytocell) showed normal signals on both chromosomes 18 (Figure 1b), the microarray (Affymetrix, Cyto 2.7) analysis confirmed the ~75 kb gain on the region (46,XY.ish subtel(41x2).mlpa (P070)x2, 18q subtel (P245)x3). arr18q23(75,516,228-75,590,562*)x3 mat). A parental array-CGH study using NimbleGen CGX-3 revealed that the phenotypically normal mother was also a carrier for the duplication (Figure 1 c,d) and the change was considered as "clinically not relevant" (46,XX. arr18q 23(75,516,228-75,590,562x3). The child was delivered at

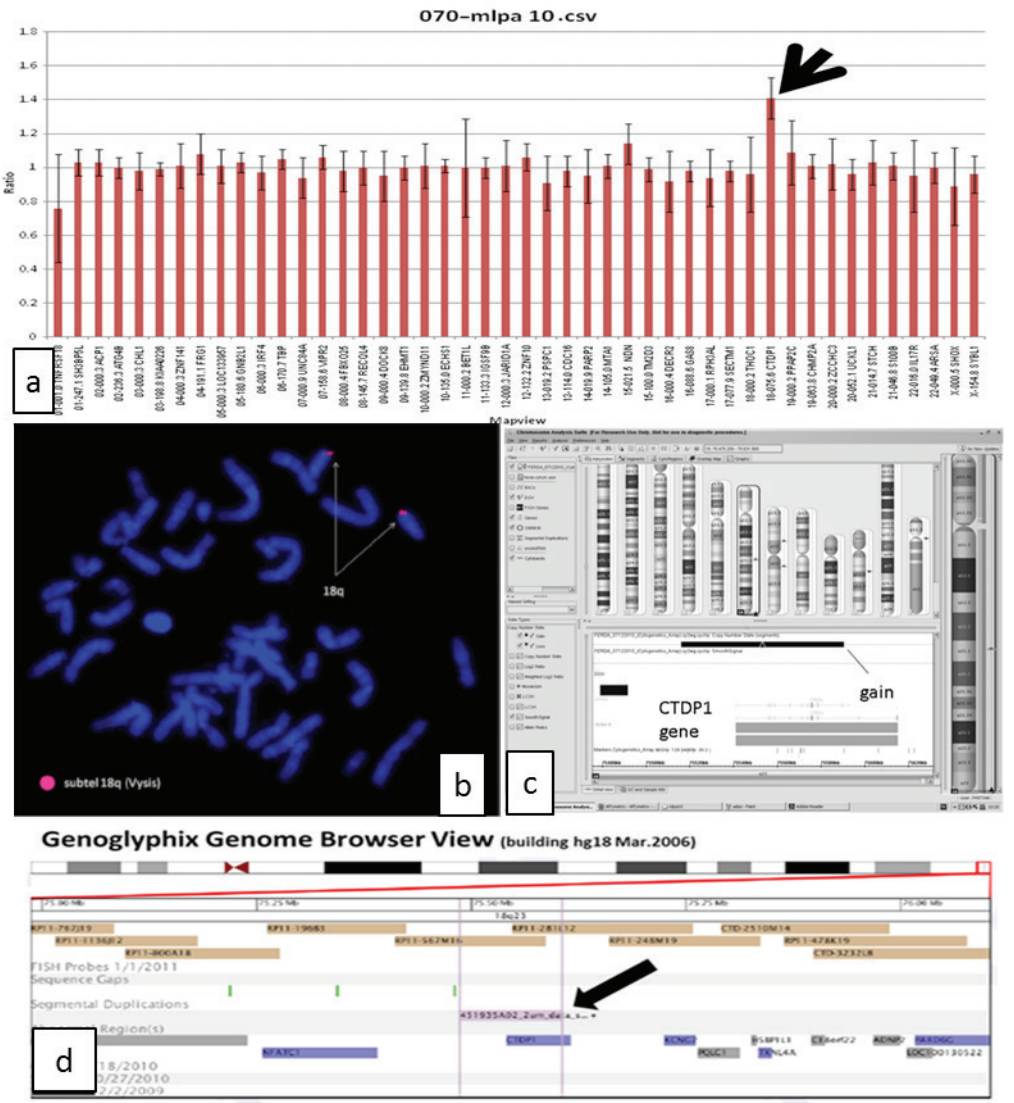

Figure 1: a) MLPA SALSA P070 probemix result ratio mapview bar graphic; for case MLPA#10, b) FISH signals with subtelomeric probe for 18q (Cytocell), c) Overlapping gained part and CTDP1 gene on Array-CGH result of the case#10, d) Array-CGH result of the mother with the same gain at the same localization.

the 34th week of gestation and died 4 days later, following surgery for the diaphragmatic hernia.

Case- 2 (MLPA#15)

The mother was referred due to the ultrasonographic finding of holoprosencephaly, single-nostril, thalamic fusion, hypotelorism, absence of nasal bone, and bilateral echogenic kidneys. At the 24th week of gestation, a fetal blood sample was obtained by fetal cord blood sampling. Routine karyotyping and subtelomeric FISH analysis revealed normal results. The pregnancy was terminated due to the possible severe, lethal outcome of antenatal USG findings.

MLPA SALSA P070 probe mix showed a deletion at subtel 18p (Figure 2a). 46,XX. ish subtel(41x2).18p subtel(P070) x1, (P245)x2. ish.del(18)(p11.3-)(D18S552-) FISH with a subtelomeric 18p probe (Telvision-Vysis) confirmed the deletion (Figure 2b), which was missed by the Multiprobe T System.

Parental cytogenetic and FISH studies revealed normal results.

Case-3 (MLPA#20)

The mother was referred at the 18th week of gestation, due to the USG findings of IUGR, short femurs, decreased dimensions of stomach and single umbilical artery. A fetal

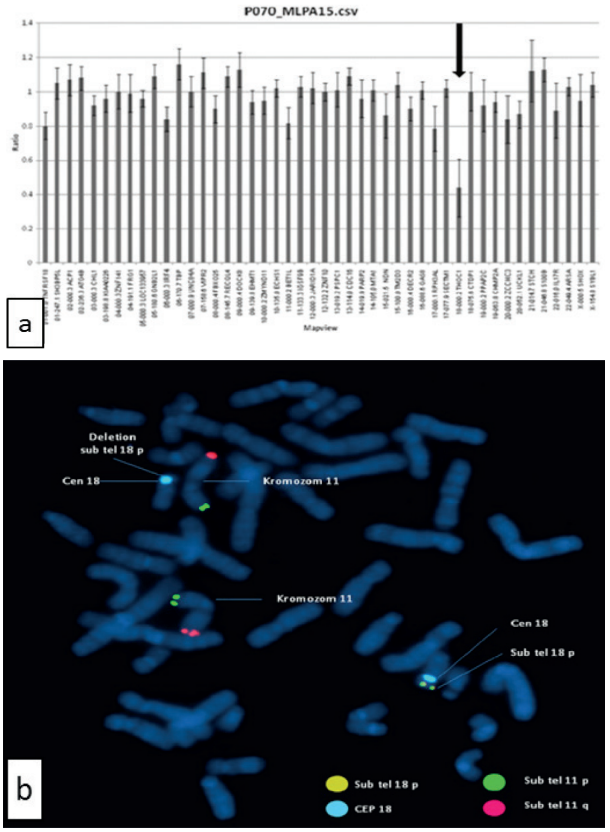

Figure 2: a) MLPA SALSA P070 probemix result ratio mapview bar graphic of case MLPA#15, b) FISH signals with subtelomeric probe for 18q (Cytocell)

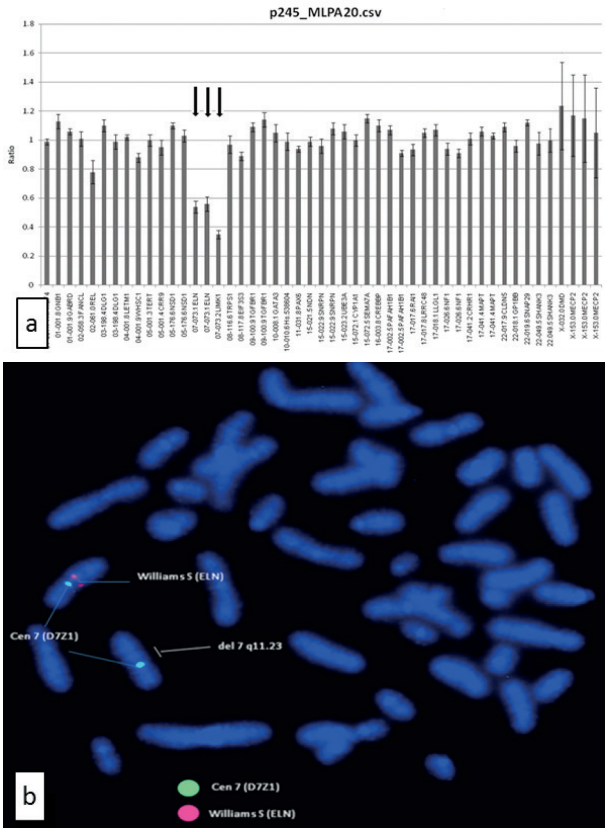

Figure 3: a) MLPA SALSA P245 probemix result ratio mapview bar graphic of case MLPA#20, b) FISH signals with subtelomeric probe of 18q (Cytocell)

blood sample was obtained by cordocentesis and routine karyotyping and subtelomeric FISH analysis revealed normal results.

A female baby was born by cesarean section due to the intrauterine fetal distress at 35 weeks of gestation. The birth weight was 1430 gr, dysmorphic features included periorbital oedema, long philtrum, thin upper lip, everted lover lip, micrognathia, pointed chin, posteriorly rotated ears and cutis marmorata. Cardiac echography revealed a small ventricular septal defect at the age of 6 months and peripheral pulmonary stenosis at the age of 14 months.

MLPA test SALSA P245 probe mix revealed a deletion on Williams Syndrome (WS) region for exon1 and exon 20 of the ELN gene and LIMK gene locus (Figure 3a) (46,XX. ish subtel(41x2). mlpa (P070)x2, 7q11.23 (P245)x1. ish. del7(q11.23-)(ELN-)). This deletion was confirmed by ELN locus specific FISH probe (Cytocell) (3b).

Parental cytogenetic and FISH studies revealed normal results.

DISCUSSION

Although the investigations carried out on children with MCA/MR underlined the importance of the subtelomeric imbalances, it is well known that the resolution of the classical karyotyping is not sufficient to detect the chromosomal imbalances of these regions. The diagnosis of the microdeletion/duplication syndromes can be ascertained in postnatal cases in the presence of distinct phenotypes for well-known syndromes. At the beginning of the 2000's, there were some reports using FISH analysis to diagnose the subtelomeric imbalances in fetuses with pathological USG findings. However, it was noted that the technique was relatively expensive and time consuming (12, 16, 22, 24). Later, MLPA was accepted as an alternative technique to identify known microdeletion/duplications and subtelomeric imbalances (14, 15, 22-24, 26).

Microarray/a-CGH techniques are able to screen the whole genome, which leads to higher detection rates, 1 to 8%, depending on the patient selection criteria and sensitivity of the test applied. These techniques are recommended, especially in the presence of pathological fetal USG findings (14, 15, 22, 25, 27).

The purpose of the study was to asses the efficacy and the impact of MLPA technique for the antenatal diagnosis in our patient cohort. In 66 fetuses with at least one major malformation with normal karyotype and subtelomeric FISH results, three chromosomal imbalances (4.5%) have been identified by MLPA. The rate of clinically relevant imbalances was 3.03% (2/66) and one imbalance was maternally inherited CNV (1.51% - 1/66). The reported rate of imbalances for MLPA in the literature varies between 0-6.5% depending on the selection criteria (16, 23, 24, 26). When specific tests are performed and the resolution of the karyotypes is high, it could be expected that the detected anomaly rate by MLPA or array studies, will be decreased (24-26). In the study from Konialis (28), MLPA was applied parallel to the karyotyping and microarray technique in all prenatal cases without any indication bias. The rate of clinically relevant imbalances was 1.2% (3/249) in cases with pathological USG findings and this rate was about 0.4% in cases with normal USG. However, in the study from Goumy (24), the rate of unbalanced rearrangements was 6.5% (4/61) in a series of cases with specific USG findings. The rate was 4.1% (33/139) in fetuses presented with major malformations and 1.6% (23/104) in fetuses with having minor, including soft markers, USG anomalies in the series of Kjaergaard (13). Our result, 3.03% in cases with major malformation is in accordance with the previously published series.

Disadvantages of the FISH analysis are the need of cell culture and high quality metaphases, which are laborious and lead to high costs. The disadvantage of the MLPA technique is that it does not cover the whole genome and multiple samples are needed to decrease the cost. Although microarray and a-CGH studies are more comprehensive and informative, the need for special equipment and consumables leads to high costs. Furthermore using experienced bioinformaticians is a must to carry out the analysis. Well known microdeletion at 7q11.23 causing WS was identified in one case. The pathological USG findings were IUGR, short femurs, decreased volume of stomach, and single umbilical artery, which were not specific for WS, and consequently FISH testing specific for WS was not performed at antenatal period. The case was retrospectively studied by MLPA using SALSA P245 probe mix, a deletion covering exon1 and 20 of ELN gene responsible for WS and LIMK gene was detected. The karyotype from the antenatal period was reanalyzed after the detection of the deletion by MLPA and no change leading to a suspicion of a deletion was observed. Clinical findings from the newborns were in accordance with WS. There are only four antenatally diagnosed WS cases in the literature, and three of them had the characteristic ultrasonographic findings of WS leading to specific testing for definite diagnosis. One case presented with VSD, and the diagnosis was established by MLPA with P064 probe mix (26), a second case reported to have supravalvular aortic stenosis (SVAS), leading to testing by FISH analysis WS specific probe (29) and the third case presented with SVAS, IUGR, interhemispheric cyst, and nasal bone hypoplasia and the deletion on 7q11.23 was identified using array-CGH (30). The fourth case reported by Lee (31), had normal USG findings and was identified using BAC array-CGH technique in the research study of 3171 consecutive prenatal samples. Our case is the second case diagnosed antenatally without any characteristic USG findings of WS. When specific fetal USG findings like SVAS, VSD are present, the FISH technique can be the first choice in the PD, otherwise MLPA or a-CGH techniques are the most powerful techniques to identify the microdeletion/duplication syndromes.

In another case, MLPA using a SALSA P070 probe mix showed a deletion on 18p, which was subsequently confirmed by subtelomeric 18p FISH prob (Telvision-Vysis). When the karyotype was reevaluated, it was considered that this microdeletion could have easily been missed by routine analysis. A false negative result in Multiprobe FISH analysis could be explained by the low-quality metaphases and false positive signals. Johnson and Bachman (32) published the first case of 18p deletion with holoprosencephaly. Gripp (33) identified the fourth gene, TGIF gene, responsible for the holoprosencephaly phenotype located on 18p at a distance of 3.5 Mb from the telomere. The distance between the TGIF gene and the THOC gene is about 3.2 Mb and SALSA P070 MLPA probe mix and subtelomeric FISH probe (Telvision-Vysis) both target the THOC gene. Therefore, it could be suggested that the deletion in our case was also covering the TGIF gene locus. There are many reports with microscopic monosomy 18p in the literature, but to our knowledge, this is the first submicroscopic deletion on 18p detected at antenatal period.

The size of the duplication on 18q telomere detected by MLPA was determined as ~75 kb by array technique. Since a phenotypically normal mother was also a carrier of the same duplication, it was interpreted as a previously unreported copy number variation (34). There were only four postnatal cases with duplication at 18q23 reported as unspecified pathogenicity in DECIPHER v5.1 (35). The gains of those cases covered the whole q arm of 18 in one, and it was a 37 Mb duplication in another. However, the duplicated region in our case was very small and only the C-terminal Domain of RNA Polymerase II subunitA, phosphatase of subunit1 (CTDP1) gene was located in this region. Homozygous loss of function mutation of the CTDP1 gene is associated with Congenital Cataracts, Facial Dysmorphism, and Neuropathy syndrome (CCFDN) in the literature (36). There is only one report presenting the gain as a polymorphic variation (37). Our findings in the family further support that the duplication of CTDP1 gene has no phenotypic effect.

Although 22q11.2 microdeletion (DiGeorge Syndrome region) is the most common submicroscopic anomaly detected by MLPA in the literature, it was not encountered in our cohort. All cases with cardiac malformations had been screened for 22q11.2 deletion by FISH analysis at the antenatal period and screened positive ones, 22q11 microdeletion cases, were not included for MLPA testing. This is the reason why this common microdeletion was not detected in our cohort.

Our study shows that MLPA is an efficient, safe, rapid and sensitive technique for the detection of submicroscopic imbalances for targeted regions of the genome. It is cost effective in comparison to FISH, since a single MLPA test can provide data for many loci concurrently for multiple samples. However, the confirmation of MLPA findings (e.g. single probe imbalances) may require FISH testing, STR analysis, qPCR or array CGH. Although, MLPA provides lower resolution than array, due to the organization of the probes, it is targeted and therefore the genotype-phenotype correlation is more predictable. The identification of de novo CNV's with uncertain significance (about 4%) leads to further testing increasing the costs, and uncertain results might also cause parental anxiety. Therefore, the testing strategy should be planned according to the relevant indication and suspected chromosomal abnormalities. If telomeric and syndrome specific analysis is sought after, MLPA can be the first-tier and the most suitable technique also at the antenatal period.

Ethics Commitee Approval: Ethics committee approval was received for this study from the ethics committee of the Istanbul Faculty of Medicine (26.09.2007_2007/2105).

I**nformed Consent:** Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- G.T., B.K., S.B., H.K., R.H.; Data Acquisition- K.Y., G.T., B.K., Z.O.U.; Data Analysis/Interpretation- G.T., B.K., K.Y., Z.O.U., P.M.; Drafting Manuscript- G.T., B.K., Z.O.U., S.B., P.M.; Critical Revision of Manuscript- G.T., B.K., P.M., S.B., Z.O.U., H.K., R.H.; Final Approval and Accountability- G.T., B.K., Z.O.U., K.Y., R.H., H.K., P.M., S.B.; Technical or Material Support- G.T., B.K., S.B., Z.O.U., H.K.; K.Y.

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REFERENCES

1. Nussbaum RL, McInnes RR, Willard HF, Hamosh A. Principles of clinical cytogenetics, Thompson&Thompson Genetics in Medicine, Saunders, Elsevier. Philadelphia, PA, 7th ed., 2007;59-113.
2. Adams-Chapman I, Hansen NI, Shankaran S, Bell EF, Boghossian NS, Murray JC, et al.; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Ten-year review of major birth defect s in VLBW infants. Pediatrics 2013;132(1):49-61. [CrossRef]
3. Eydoux P, Choiset A, Le Porrier N, Thépot F, Szpiro-Tapia S, Alliet J, et al. Chromosomal prenatal diagnosis: study of 936 cases of intrauterine abnormalities after ultrasound assessment. Prenat Diagn 1989;9(4):255-69. [CrossRef]
4. Daniel A, Athayde N, Ogle R, George AM, Michael J, Pertile MD, et al. Prospective ranking of the sonographic markers for aneuploidy: data of 2143 prenatal cytogenetic diagnoses referred for abnormalities on ultrasound. Aust N Z J Obstet Gynaecol 2003;43(1):16-26. [CrossRef]
5. Zhang L, Zhang XH, Liang MY, Ren MH. Prenatal cytogenetic diagnosis study of 2782 cases of high-risk pregnant women. Chin Med J (Engl) 2010;123(4):423-30.
6. Lichtenbelt KD, Alizadeh BZ, Scheffer PG, Stoutenbeek P, Schielen PC, Page-Christiaens LC, et al. Trends in the utilization of invasive prenatal diagnosis in The Netherlands during 2000-2009. Prenat Diagn 2011;31(8):765-72. [CrossRef]
7. D'Amours G, Kibar Z, Mathonnet G, Fetni R, Tihy F, De´silets V, et al. Whole-genome array CGH identifies pathogenic copy number variations in fetuses with major malformations and a normal karyotype. Clin Genet 2012;81(2):128-41. [CrossRef]
8. Flint J, Wilkie AO, Buckle VJ, Winter RM, Holland AJ, McDermid HE. The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. Nat Genet 1995;9(2):132-40. [CrossRef]
9. Knight SJ, Regan R, Nicod A, Horsley SW, Kearney L, Homfray T, et al. Subtle chromosomal rearrangements in children with unexplained mental retardation. Lancet 1999;354(9191):1676-81. [CrossRef]
10. Rossi E, Piccini F, Zollino M, Neri G, Caselli D, Tenconi R, et al. Cryptic telomeric rearrangements in subjects with mental retardation associated with dysmorphism and congenital malformations. J Med Genet 2001;38(6):417-20. [CrossRef]

11. Baker E, Hinton L, Callen DF, Altree M, Dobbie A, Eyre HJ, et al. Study of 250 children with idiopathic mental retardation reveals nine cryptic and diverse subtelomeric chromosome anomalies. Am J Med Genet 2002;107(4):285-93. [CrossRef]
12. Gignac J, Danis K, Tihy F, Lemyre E. Prenatal detection of subtelomeric rearrangements by multi-subtelomere FISH in a cohort of fetuses with major malformations. Am J Med Genet A 2006;140(24):2768-75. [CrossRef]
13. Kjaergaard S, Sundberg K, Jørgensen FS, Rohde MD, Lind AM, Gerdes T, et al. Diagnostic yield by supplementing prenatal metaphase karyotyping with MLPA for microdeletion syndromes and subtelomere imbalances. Prenat Diagn 2010;30(10):995-9. [CrossRef]
14. Shaffer LG, Bejjani BA. A cytogeneticist's perspective on genomic microarrays. Hum Reprod Update 2004;10(3):221-6. [CrossRef]
15. Shaffer LG, Coppinger J, Alliman S, Torchia BA, Theisen A, Ballif BC, et al. Comparison of microarray-based detection rates for cytogenetic abnormalities in prenatal and neonatal specimens. Prenat Diagn 2008;28(9):789-95. [CrossRef]
16. Schou KV, Kirchhoff M, Nygaard U, Jorgersen C, Sundberg K. Increased nuchal translucency with normal karyotype: afollow-up study of 100 cases supplemented with CGH and MLPA analyses. Ultrasound Obstet Gynecol 2009;34(6):618-22. [CrossRef]
17. Schaffer LG, Bejjani BA. Medical applications of array CGH and the transformation of clinical cytogenetics. Cytogenet Genome Res 2006;115(3-4):303-9. [CrossRef]
18. Vialard F, Molina Gomes D, Leroy B, Quarello E, Escalona A, Le Sciellour C, et al. Array comparative genomic hybridization in prenatal diagnosis: another experience. Fetal Diagn Ther 2009;25(2):277-84. [CrossRef]
19. Thuresson AC, Bondeson ML, Edeby C, Ellis P, Langford C, Dumanski JP, et al. Whole-genome array-CGH for detection of submicroscopic chromosomal imbalances in children with mental retardation. Cytogenet Genome Res 2007;118(1):1-7. [CrossRef]
20. Slavotinek AM. Novel microdeletion syndromes detected by chromosome microarrays. Hum Genet 2008;124(1):1-17. [CrossRef]
21. Engels H, Wohlleber E, Zink A, Hoyer J, Ludwig KU, Brockschmidt FF, et al. A novel microdeletion syndrome involving 5q14.3-q15: clinical and molecular cytogenetic characterization of three patients. Eur J Hum Genet 2009;17(12):1592-9. [CrossRef]
22. Gross SJ, Bajaj K, Garry D, Klugman S, Karpel BM, Marie Roe A, et al. Rapid and novel prenatal molecular assay for detecting aneuploidies and microdeletion syndromes. Prenat Diagn 2011;31(3):259-66. [CrossRef]
23. Mademont-Soler I, Morales C, Bruguera C, Madrigal I,Clusellas N, Margarit E, et al. Subtelomeric MLPA: is it really useful in prenatal diagnosis? Prenat Diagn 2010;30(12-13):1165-9. [CrossRef]
24. Goumy C, Gouas L, Pebrel-Richard C, Véronèse L, Eymard-Pierre E, Debost-Legrand A, et al. Prenatal detection of cryptic rearrangements by multiplex ligation probe amplification in fetuses with ultrasound abnormalities. Genet Med 2010;12(6):376-80. [CrossRef]
25. Kleeman L, Bianchi DW, Shaffer LG, Rorem E, Cowan J, Craigo SD, et al. Use of array comparative genomic hybridization for prenatal diagnosis of fetuses with sonographic anomalies and normal metaphase karyotype. Prenat Diagn 2009;29(13):1213-7. [CrossRef]
26. Kontos H, Manolakos E, Malligiannis P, Plachouras N, Ploumis N, Mihalatos M, et al. Prenatal diagnosis of a fetus with 7q11.23 deletion detected by multiplex ligation-dependent probe amplification (MLPA) screening. Prenat Diagn 2008;28(6):556-8. [CrossRef]
27. Van den Veyver IB, Patel A, Shaw CA, Pursley AN, Kang SH, Simovich MJ, et al. Clinical use of array comparative genomic hybridization (aCGH) for prenatal diagnosis in 300 cases. Prenat Diagn 2009;29(1):29-39. [CrossRef]
28. Konialis C, Hagnefelt B, Sevastidou S, Karapanou S, Pispili K, Markaki A, et al. Uncovering recurrent microdeletion syndromes and subtelomeric deletions/duplications through non-selective application of a MLPA based extended prenatal panel in routine prenatal diagnosis. Prenat Diagn 2011;31(6):571-7. [CrossRef]
29. Krzeminska D, Steinfeld C, Cloez JL, Vibert M, Chery M, Menziesat D, et al. Prenatal diagnosis of Williams syndrome based on ultrasound signs. Prenat Diagn 2009;29(7):710-2. [CrossRef]
30. Popowski T, Vialard F, Leroy B, Bault JP, Molina-Gomes D. Williams-Beuren syndrome: the prenatal phenotype. Am J Obstet Gynecol 2011;205(6):e6-8. [CrossRef]
31. Lee CN, Lin SY, Lin CH, Shih JC, Lin TH, Su YN. Clinical utility of array comparative genomic hybridization for prenatal diagnosis:a cohort study of 3171 pregnancies. BJOG 2012; 119(5):614–25. [CrossRef]
32. Johnson G, Bachman R. A 46,XY,del(18)(pter-p1100:) cebocephalic child from a 46,XX,t(12;18)(18pter-18p1100::12qter-12pter) normal parent. Hum Genet 1976;34(1):103-6. [CrossRef]
33. Gripp KW, Wotton D, Edwards MC, Roessler E, Ades L, Meinecke P, et al. Mutations in TGIF cause holoprosencephaly and link NODAL signalling to human neural axis determination. Nat Genet 2000;25(2):205-8. [CrossRef]
34. Database of genomic variation, "A curated catalogue of human genomic structural variation", 2018/09/21, Available from: http://dgv.tcag.ca/dgv/app/variant?id=dgv3407n100&ref=GRCh37/hg19
35. DECIPHER, "Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources", 2018/09/21 Available from: https://decipher.sanger.ac.uk/patient/291627#genotype/cnv/67391/browser/18:76996166-77946488
36. Varon R, Gooding R, Steglich C, Marns L, Tang H, Angelicheva D et al. Partial deficiency of the C-terminal-domain phosphatase of RNA polymerase II is associated with congenital cataracts facial dysmorphism neuropathy syndrome. Nat Genet 2003;35(2):185-9. [CrossRef]
37. Choi JS, Lee WJ, Baik SH, Yoon HK, Lee KH, Kim YH. Array CGH reveals genomic aberrations in human emphysema. Lung 2009;187(3):165-72. [CrossRef]