Multiplex Ligation-dependent Probe Amplification (MLPA) – new possibilities of prenatal diagnosis

Multiplex Ligation-dependent Probe Amplification (MLPA) – nowe możliwości diagnostyki prenatalnej

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Abstract

Multiplex Ligation-dependent Probe Amplification (MLPA) is a relatively new method of molecular diagnosis. It enables a relative quantitative assessment of up to 50 different PCR amplicons in one reaction by the use of a very small amount of examined DNA. Nowadays MLPA is becoming a very helpful tool in prenatal diagnosis and is widely used for the detection of aneuploidies, familial single gene disorders, common microdeletion syndromes, sub-telomeric alterations and identification of marker chromosomes in fetuses. This review demonstrates possible applications of MLPA in prenatal diagnosis.

Key words: **Multiplex Ligation-dependent Probe Amplification (MLPA)** / / **prenatal diagnosis** / **aneuploidy detection** /

Streszczenie

Multiplex Ligation-dependent Probe Amplification (MLPA) jest stosunkowo nową metodą diagnostyki molekularnej. Umożliwia względną ilościową ocenę do 50 różnych amplikonów PCR w jednej reakcji, przy użyciu bardzo małej ilości badanego DNA. Obecnie MLPA staje się bardzo użytecznym narzędziem diagnostyki prenatalnej i jest szeroko stosowane do wykrywania aneuploidii, rodzinnych przypadków chorób monogenowych, zespołów mikrodelecji, zmian subtelomerowych i identyfikacji chromosomów markerowych u płodów. Praca ta prezentuje możliwości zastosowania MLPA w diagnostyce prenatalnej.

Słowa kluczowe: **Multiplex Ligation-dependent Probe Amplification (MLPA)** / / **diagnostyka prenatalna** / **wykrywanie aneuploidii** /

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Introduction

Multiplex Ligation-dependent Probe Amplification (MLPA) was introduced in 2002 by MRC Holland as a method of a relative quantitative assessment of the copy number changes in DNA. Currently, there are over 300 different assays of MLPA available for genomic DNA screening and two special variations: RT – MLPA for mRNA and MS – MLPA for methylation profiling [1].

The advantages of MLPA include: a very short time to diagnosis (2-4 days), effectiveness, simplicity and relatively low costs. MLPA is a useful tool for the detection of chromosomal numerical aberrations - chromosome 13, 18, 21, X and Y aneuploidies [2, 3], but it also enables to identify changes as small as deletions or duplications of single exons in selected genes [4].

Pregnant women with an increased risk for chromosomal aberrations diagnosed on the basis of non-invasive screening in the first trimester in accordance with the standards of the Fetal Medicine Foundation (the maternal age, the nuchal translucency thickness measurement, the fetal nasal bone assessment combined with maternal serum biochemical markers – PAPP-A and free beta HCG) [5, 6] and women with a familial history of single gene disorders should undergo a first trimester (11-13 gestation weeks) chorionic villus sampling (CVS) and molecular testing [7]. In these cases, MLPA enables a diagnosis in just a few days, which is crucial if a genetic abnormality is confirmed and further course of the pregnancy has to be determined.

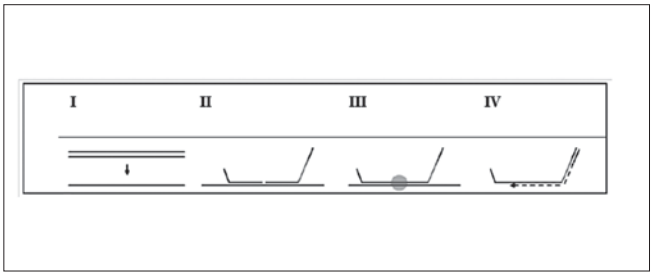

Figure 1. Schematic diagram of MLPA reaction phases (according to www.mlpa.com):
I – DNA sample denaturation; II – hybridization of the probes to the complementary target sequences; III – probes ligation; IV – PCR amplification of ligated probes.

Abnormal findings detected in the second trimester scan in a chromosomally normal fetus are an indication for further testing for common microdeletion syndromes and subtelomeric alterations, that may be performed using MLPA on stored DNA obtained by CVS in the first and amniocentesis or cordocentesis in the second trimester of pregnancy [8, 9].

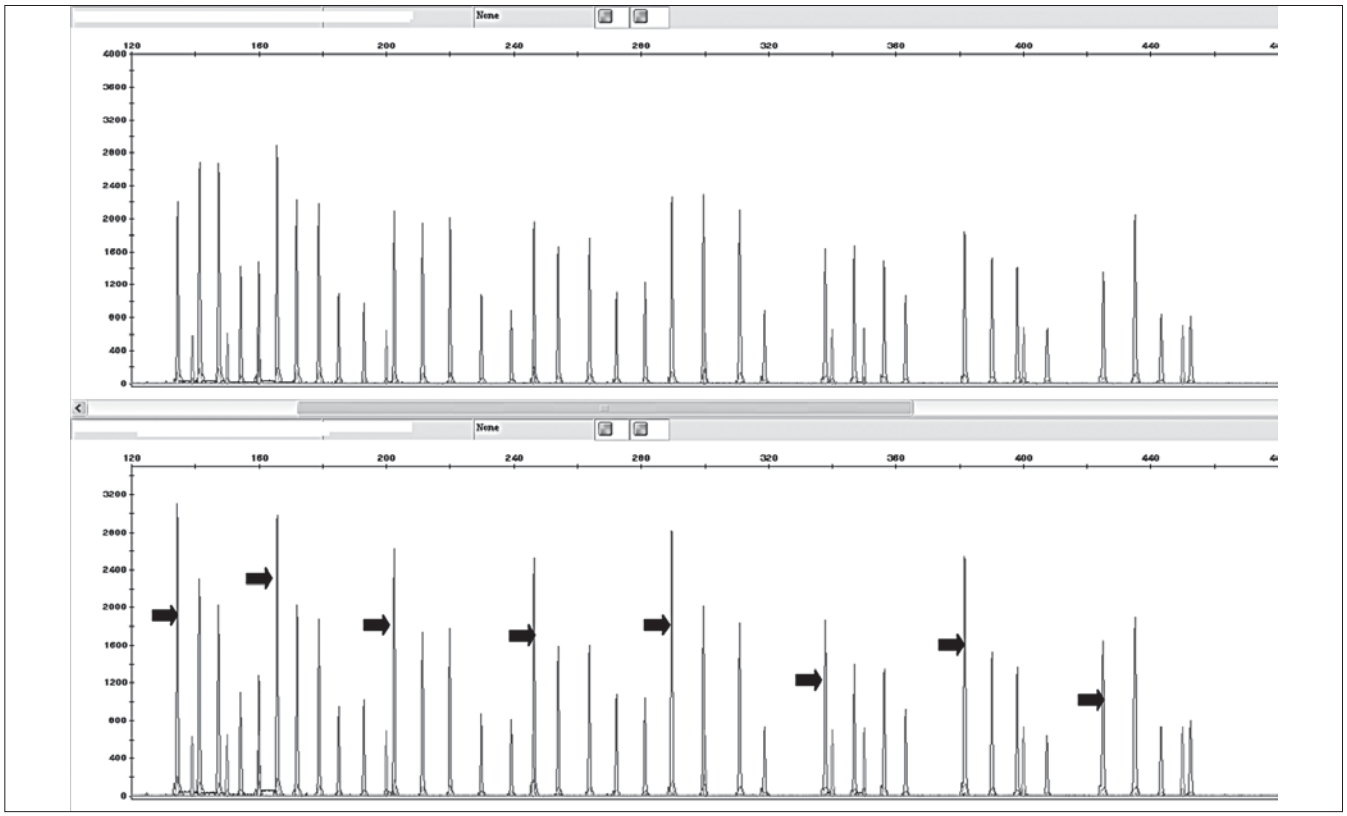

Figure 2. Results of capillary electrophoresis and data analysis of the MLPA assay for 21, 18, 13, X, Y aneuploidies.

Upper: Healthy control. Visible picks of tested regions on the chromosomes: 21, 18, 13, X and Y.
Lower: 21 trisomy (Down syndrome). Visible normal picks of the tested regions on chromosomes: 18, 13, X and Y. Significantly higher picks of chromosome 21 (black arrows) in comparison with other picks in the same test and in healthy controls.

Pictures obtained from the Genetics Department of the Institute of Psychiatry and Neurology, Warsaw.

Description of the method

MLPA is based on the hybridization and PCR method, but some improvements have been introduced for its higher effectiveness, simplicity and sensitivity.

MLPA enables a relative quantitative assessment of up to 50 different PCR amplicons in one reaction, completed in 24-48 hours. As it is the ligated probe and not the tested DNA itself that undergoes multiplication, only 50-250 ng of genomic DNA are needed for one reaction.

MLPA reaction is divided into the following phases: DNA sample denaturation, hybridization of the probes to the complementary target sequences, probes ligation, PCR amplification of the ligated probes and capillary electrophoresis of the products (figure 1). An analysis of the results may be performed afterwards (figure 2).

For each examined sequence two oligonucleotide probes, that are complementary to adjacent regions, are designed. After hybridization to DNA (annealing), ligation of the adjacent bound probes occurs. Only after ligation is the probe ready for PCR reaction. Shorter – the left probe oligonucleotide (LPO) contains a primer binding sequence at its 5' end. Longer – the right probe oligonucleotide (RPO) contains other primer binding sequence at the 3' end and also a "stuffer" sequence, which has different length in each probe (figure 3). Only one primer pair is used for all probes and products of different length are obtained.

The PCR products are separated through capillary electrophoresis. The results are analyzed by comparison with healthy controls or with the internal control fragments (figure 2). Peak ratios between 0.7 – 1.3 are considered normal. Ratios higher than 1.3 indicate a gain of the examined sequence, ratios lower than 0.7 indicate a loss of the target [1].

Detection of aneuploidy

One year after the MLPA method was introduced into the postnatal diagnosis in 2002, the first steps of its prenatal usage were undertaken. In 2003, the first report of antenatal detection of chromosomes 13, 18, 21 , X and Y aneuploidies was published [2]. MLPA results were compared to standard karyotyping and FISH results and complete concordance was confirmed.

Many further studies showed similar compliance of the MLPA and standard karyotyping in the detection of full trisomies for chromosomes: 13 (Patau syndrome), 18 (Edwards syndrome), 21 (Down syndrome) and sex chromosome imbalances – X monosomy (Turner syndrome), 47, XXY (Klinefelter syndrome) and 47, XYY (Jakobs syndrome) in chorionic villus samples and amniotic fluid cells [10-12].

However, in cases of small chromosomal changes: structural rearrangements or abnormalities of the regions uncovered by the MLPA probes, in 0.6 to 2.4% the results between karyotyping and MLPA may be discordant [10, 12, 13]. In general, MLPA is also unable to detect triploidy (69, XXX and 69, XXY) [14], but some cases of detecting 69, XXY triploidy by means of MLPA were reported [15].

Maternal cell contamination (MCC), especially in female fetuses, may cause interpretation bias of the MLPA results. Therefore, MCC studies are recommended to be performed in conjunction with MLPA [16].

Mosaicism denotes the presence of two different cell lines in one individual. It is especially significant in testing of chorionic villi, where it can be present in up to 1% of all samples, due to placental mosaicism (mosaicism limited to the placenta) [17]. MLPA is unable to exclude low-level placental and true mosaicism [18]. A detection of the more clinically relevant high-level mosaicism (with the fraction of abnormal cells of 20-30%) has been documented [11, 12].

Detection of subtelomeric alterations, microdeletions and marker chromosomes

In cases with prenatal abnormal ultrasound findings and normal karyotype, a more detailed molecular testing may be recommended. MLPA enables testing of the centromeres, subtelomeric regions, and regions with recurrent microdeletions for aberrations that are responsible for rarely occurring syndromes, such as DiGeorge, Prader-Willi/Angelman, Miller-Dieker, Williams or Smith-Magenis syndrome.

Although in previous MLPA studies relatively few molecular abnormalities were detected (from 0% in fetuses with abnormal first trimester screening, normal karyotype and no additional ultrasound findings [3] to 4.1% in cases with structural malformations [19], in cases with multiple abnormal ultrasound findings or abnormalities characteristic for a specific genetic syndrome, MLPA screening may be useful.

MLPA may also be successfully used as a method for the follow-up of karyotypes in which small supernumerary marker chromosomes were detected, enabling the identification of their origin [20].

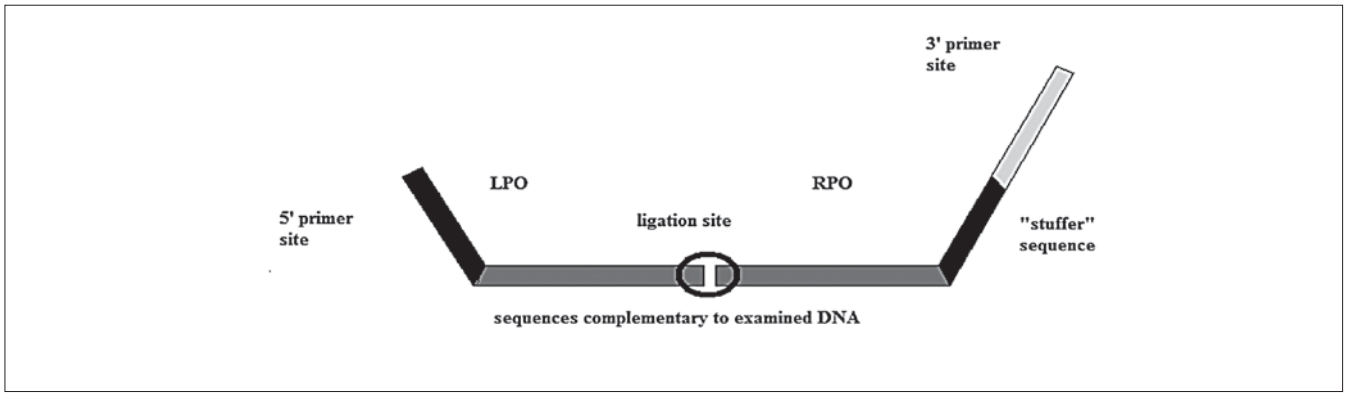

Figure 3. Schematic diagram of the MLPA probe structure. (LPO – left probe oligonucleotide; RPO - right probe oligonucleotide)

Detection of single gene disorders

MLPA is a very useful tool for a quick prenatal detection of single gene disorders such as congenital adrenal hyperplasia, tuberous sclerosis, Duchenne/Becker muscular dystrophy and spinal muscular atrophy in cases with a known family history and identified familial mutation [21-24].

The advantages of MLPA over other methods (PCR-multiplex, FISH) include a possibility of the whole gene examination (all exons) in one or two reactions, the detection of exact boundaries of the mutation, as well as a simple identification of deletions and duplications [25].

MLPA enables also a prenatal detection of the female carriers of X-linked diseases, and carriers of recessive autosomal disorders, though for ethical reasons it is not recommended [26].

Conclusions

MLPA is a relatively new, cost effective method of comparative quantitative molecular testing, that may have a wide application in prenatal diagnosis.

Its most significant advantage is the possibility of a rapid molecular diagnosis, which is especially important in cases with serious ultrasound findings and a high risk for chromosomal aberrations. It enables a rapid reassurance of parents and earlier decisions regarding further pregnancy management.

As for one MLPA reaction, only 50-250 ng of genomic DNA is sufficient [1], there is no need for cell culture and the diagnosis can be performed even if a very small amount of the material is provided.

In chromosomally normal fetuses with severe structural malformations or intrauterine growth restriction (IUGR) further genetic testing is indicated [27, 28] and MLPA assays for microdeletion syndromes and subtelomeric alterations may be useful.

The effectiveness of MLPA in postnatal diagnosis of single gene disorders is widely documented [4, 26]. Although there are only few reports of MLPA usage prenatally, it seems a promising method and has a broad application in the prenatal detection of single gene disorders.

Due to MLPA imperfection in low-grade mosaicism and triploidy detection, it seems justified to complement MLPA with standard karyotyping, which lasts longer, but may contribute new information in cases where MLPA diagnosis is not possible.

Despite its limitations, MLPA has a great potential for prenatal molecular diagnosis and its applications should be known to gynecologists performing noninvasive and invasive prenatal screening.

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