

Research Letter

Rapid aneuploidy diagnosis by multiplex ligation-dependent probe amplification using uncultured amniocytes in pregnancy with major fetal structural abnormalities

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Case 1. A 41-year-old woman (gravida 1) underwent amniocentesis at 17 weeks of gestation because of advanced maternal age. Prenatal ultrasound revealed nuchal edema, nasal hypoplasia, and endocardial cushion defects (Fig. 1). Aspiration of amniotic fluid yielded 8 mL with uncultured amniocytes that was applied for multiplex ligation-dependent probe amplification (MLPA) using the SALSA MLPA P095-A2 aneuploidy kit (MRC-Holland bv, Amsterdam, The Netherlands) to detect aneuploidies of chromosomes X, Y, 13,

18, and 21; 8 mL with uncultured amniocytes was applied for quantitative fluorescent polymerase chain reaction (QF-PCR); and 16 mL was applied for conventional cytogenetic analysis using cell culture of the amniocytes. Within 2 days, MLPA showed the result of a female fetus with three copies of all targets on chromosome 21, and two copies of all targets on chromosomes X, 13, and 18 within the P095-A2 kit [mlpa X (P095-A2)×2, 13 (P095-A2)×2, 18 (P095-A2)×2, 21 (P095-A2)×3] (Fig. 2). QF-PCR analysis confirmed the prenatal



Fig. 1. Prenatal ultrasound in Case 1 shows (A) nasal hypoplasia, (B) nuchal edema, and (C) endocardial cushion defects.

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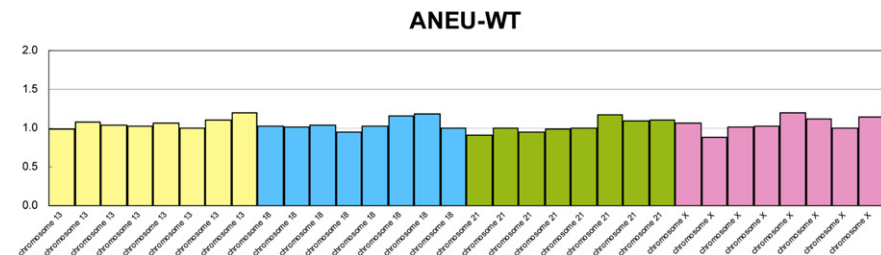
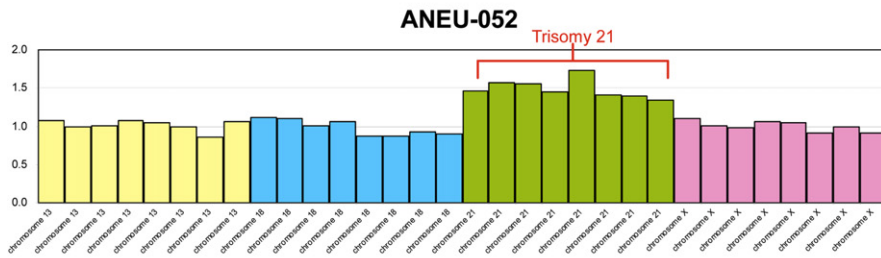
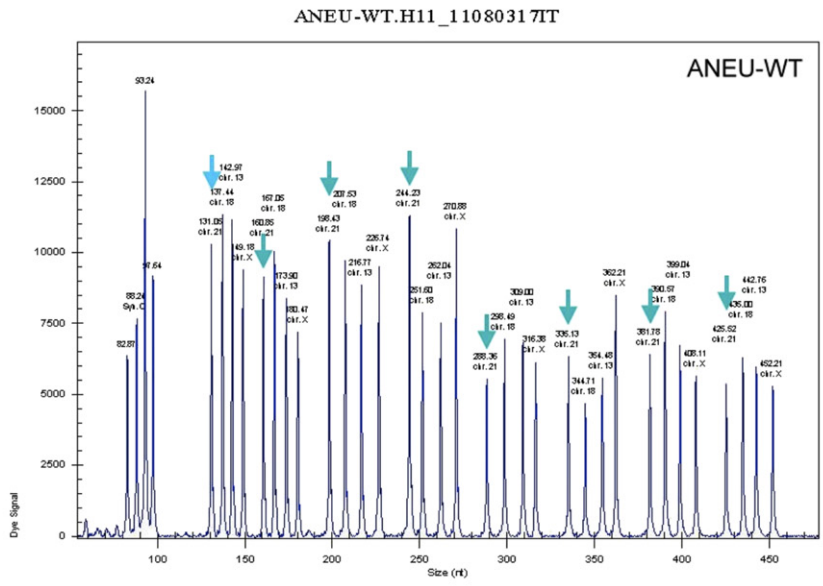
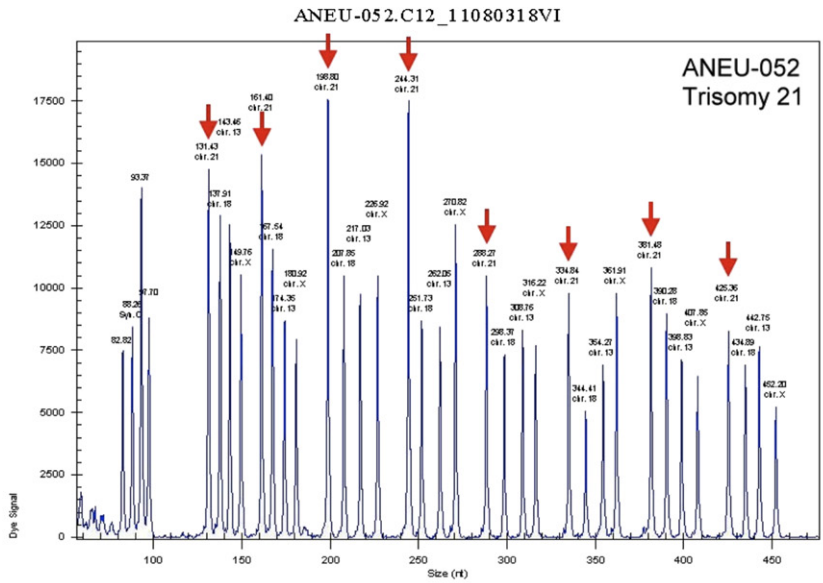


Fig. 2. Graphic analysis of multiplex ligation-dependent probe amplification (MLPA) in Case 1. The female fetus has three copies of all targets on chromosome 21 consistent with the diagnosis of trisomy 21. The wild type in a normal female control has two copies of all targets on chromosome 21. Arrows indicate chromosome 21 targets. ANEU = aneuploidy test; ANEU-052 = Case 1; ANEU-WT = wild type (control); WT = wild type.

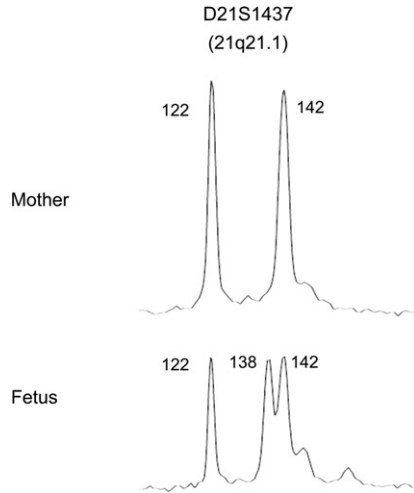


Fig. 3. Representative electrophoretograms of quantitative fluorescent polymerase chain reaction (QF-PCR) assays in Case 1 using the informative marker D21S1437 (21q21.1) show three peaks (122 bp:138 bp:142 bp; maternal:paternal:maternal) of three different parental alleles of equal fluorescent activity with a dosage ratio of 1:1:1 in uncultured amniocytes, indicating a maternal origin of the extra chromosome 21 and a possible maternal meiosis I nondisjunction.



Fig. 4. Photograph of the fetus in Case 1. Nuchal edema, nasal hypoplasia, and low-set ears are seen at birth.

diagnosis and maternal origin of the extra chromosome 21 (Fig. 3). Conventional cytogenetic analysis revealed a karyotype of 47,XX,+21. The pregnancy was subsequently terminated, and a 200-g fetus with facial dysmorphism and nuchal edema was delivered at 18 weeks of gestation (Fig. 4).

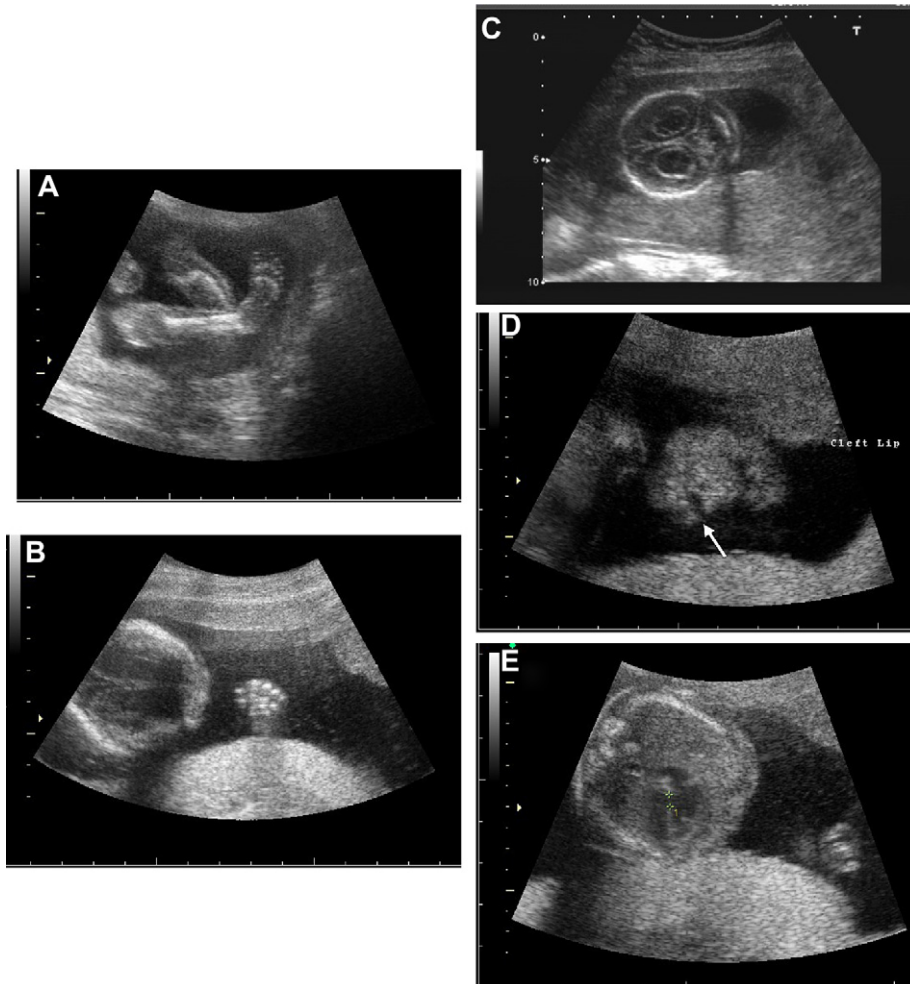


Fig. 5. Prenatal ultrasound of Case 2 shows (A) left club foot, (B) clenched hand, (C) choroid plexus cysts, (D) facial cleft (arrow), and (E) ventricular septal defect.

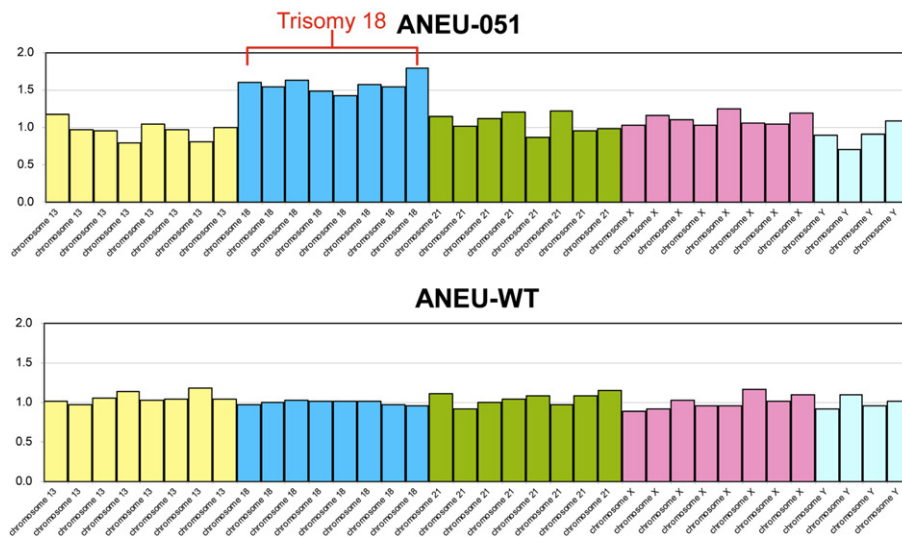
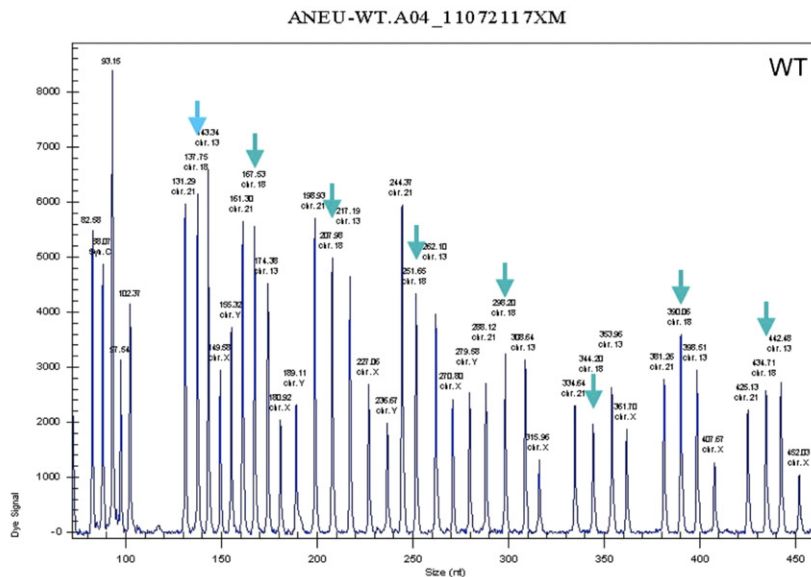
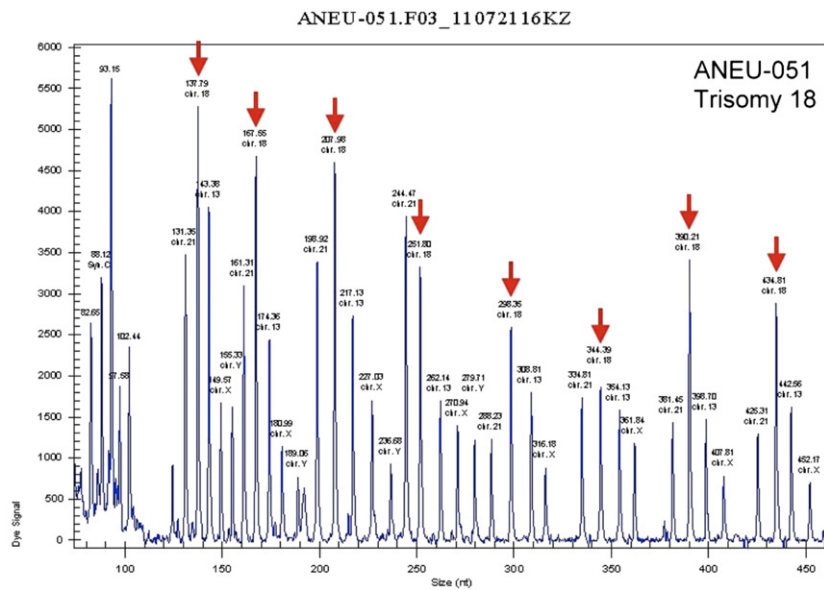


Fig. 6. Graphic analysis of multiplex ligation-dependent probe amplification (MLPA) in Case 2. The male fetus has three copies of all targets on chromosome 18 consistent with the diagnosis of trisomy 18. The wild type in a normal male control has two copies of all targets on chromosome 18. Arrows indicate chromosome 18 targets. ANEU = aneuploidy test; ANEU-051 = Case 2; ANEU-WT = wild type (control); WT = wild type.

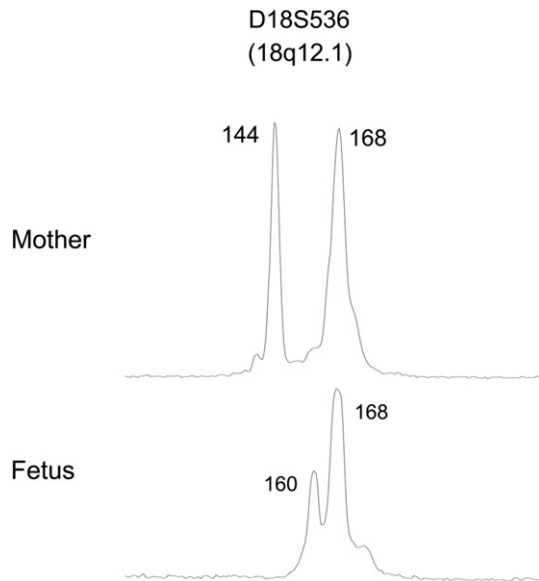


Fig. 7. Representative electrophoretograms of QF-PCR assays in Case 2 using the informative marker D18S536 (18q12.1) show two peaks (160 bp:168 bp; paternal:maternal) of two different parental alleles of unequal fluorescent activity with a dosage ratio of 1:2 (paternal:maternal) in uncultured amniocytes, indicating a maternal origin of the extra chromosome 18 and a possible maternal meiosis II nondisjunction.

Case 2. A 40-year-old woman (gravida 5, para 2) underwent amniocentesis at 18 weeks of gestation because of advanced maternal age. Prenatal ultrasound revealed choroid plexus cysts, cleft lip and palate, ventricular septal defect, clubfoot, and clenched hands (Fig. 5). Aspiration of amniotic

fluid yielded 10 mL with uncultured amniocytes that was applied for MLPA, 8 mL with uncultured amniocytes applied for QF-PCR, and 18 mL applied for cell culture and conventional cytogenetic analysis. Within 2 days, MLPA showed the result of a male fetus with three copies of all targets on chromosome 18, two copies of all targets on chromosomes 13 and 21, and one copy of all targets on chromosomes X and Y within the P095-A2 kit [mlpa X,Y (P095-A2)×1, 13 (P095-A2)×2, 18 (P095-A2)×3, 21 (P095-A2)×2] (Fig. 6). QF-PCR analysis confirmed the prenatal diagnosis and maternal origin of the extra chromosome 18 (Fig. 7). Conventional cytogenetic analysis revealed a karyotype of 47,XY,+18. The pregnancy was subsequently terminated, and a 260-g fetus with facial cleft, left clubfoot, and clenched hands was delivered at 19 weeks of gestation (Fig. 8).

Common aneuploidies such as trisomy 21, trisomy 18, trisomy 13, 45,X, 47,XXY, 47,XXX, 47,XYY, 69,XXX, and 69,XXY account for more than 80% of chromosomal abnormalities at prenatal diagnosis [1]. Rapid aneuploidy diagnosis (RAD) refers to applications of interphase fluorescence *in situ* hybridization (FISH), MLPA, QF-PCR, or array-based comparative genomic hybridization (aCGH) for rapid prenatal diagnosis of common aneuploidies [1]. Schouten et al [2] first described the application of MLPA in molecular diagnostics. MLPA is a molecular method to detect gene dosage abnormalities in a wide range of conditions by relative quantification of multiple DNA target sequences in one PCR with the input of 20 ng or more DNA but without the requirement of living cells or cell cultures [2–4]. The MLPA kits for RAD are commercially available, such as SALSA MLPA

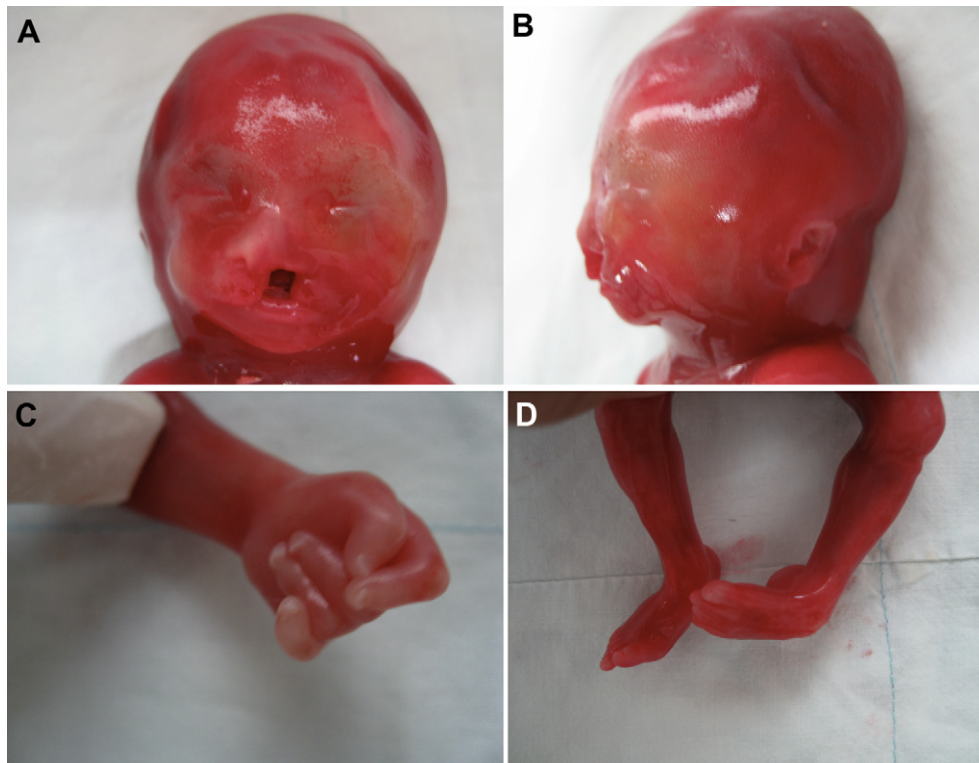


Fig. 8. The fetus in Case 2 presents with (A) cleft lip and palate and hypertelorism, (B) low-set ears, (C) clenched hand, and (D) clubfoot at birth.

P181-A2 and P181-B1 centromere kits for rapid detection of marker chromosomes [5], and SALSA MLPA P095 aneuploidy kit for rapid detection of common aneuploidies [6]. For the SALSA MLPA P095 aneuploidy kit, eight target sequences are chosen for each of the chromosomes 13, 18, 21, and X, and four target sequences are chosen for chromosome Y. MLPA has the advantages of rapid analysis of target chromosomal abnormalities with a relatively lower cost but without the need of cell culture [2,7,8]. MLPA has the disadvantages of the inability to detect structural chromosomal abnormalities, maternal cell contamination, low-level mosaicism, and 69,XXX, although Van Opstal et al [4] recently have been able to improve the detection rate of mosaicism, structural chromosomal abnormalities, and triploidy by calculating the cutoff values for all the probes in the P095 MLPA kit and comparing the relative probe signals with the normal cutoff values.

Our first case was associated with nuchal edema, nasal hypoplasia, endocardial cushion defects, and trisomy 21. Our second case was associated with choroid plexus cysts, cleft lip and palate, ventricular septal defect, clubfoot, clenched hands, and trisomy 18. Both cases had RAD by MLPA using uncultured amniocytes. RAD by aCGH using uncultured amniocytes in pregnancy with fetal structural abnormalities has been well described [9]. This presentation highlights the usefulness of the MLPA P095-A2 aneuploidy kit in RAD of common aneuploidies by using uncultured amniocytes at amniocentesis in pregnancy with major fetal structural abnormalities.

Acknowledgments

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