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Case Report

Rapid aneuploidy diagnosis by multiplex ligation-dependent probe amplification and array comparative genomic hybridization in pregnancy with major congenital malformations

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Abstract

Objective: To report five cases of major congenital malformations associated with common aneuploidies detected by rapid aneuploidy diagnosis.
Case Reports: The fetus in the first case presented cebocephaly, semilobar holoprosencephaly, and tetralogy of Fallot on ultrasound at 25 gestational weeks. Cordocentesis using multiplex ligation-dependent probe amplification to detect aneuploidies of chromosomes X, Y, 13, 18, and 21 in uncultured cord blood revealed three copies of all targets on chromosome 13 consistent with the diagnosis of trisomy 13. The fetus in the second case presented bilateral choroid plexus cysts, congenital diaphragmatic hernia, and club foot on ultrasound at 18 gestational weeks. Amniocentesis using array-based comparative genomic hybridization (aCGH) in uncultured amniocytes revealed a gain in the DNA dosage of chromosome 18 consistent with the diagnosis of trisomy 18. The fetus in the third case presented aortic stenosis and nuchal edema on ultrasound at 22 gestational weeks. Amniocentesis using aCGH in uncultured amniocytes revealed a result of monosomy X and Turner syndrome. The fetus in the fourth case presented nuchal cystic hygroma and ventriculomegaly on ultrasound at 17 gestational weeks. Amniocentesis using aCGH in uncultured amniocytes revealed a gain in the DNA dosage of chromosome 21 consistent with the diagnosis of trisomy 21. The fetus in the fifth case presented holoprosencephaly, omphalocele, and hydronephrosis on ultrasound at 17 gestational weeks. Amniocentesis using aCGH in uncultured amniocytes revealed a gain in the DNA dosage of chromosome 13 consistent with the diagnosis of trisomy 13.
Conclusions: Prenatal diagnosis of major congenital malformations should alert one to the possibility of chromosomal abnormalities. Multiplex ligation-dependent probe amplification and aCGH have the advantage of rapid aneuploidy diagnosis of common aneuploidies in cases with major congenital malformations.

Keywords: Array-based comparative genomic hybridization (aCGH); Multiplex ligation-dependent probe amplification (MLPA); Rapid aneuploidy diagnosis

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Introduction

Rapid aneuploidy diagnosis (RAD) refers to the applications of molecular cytogenetic techniques such as interphase fluorescence *in situ* hybridization, quantitative fluorescent polymerase chain reaction, multiplex ligation-dependent probe amplification (MLPA), and array-based comparative genomic hybridization (aCGH) for rapid prenatal diagnosis of common aneuploidies [1]. Common aneuploidies, such as trisomies 13, 18 and 21, Turner syndrome (45,X), Klinefelter syndrome (47,XXY), triple X (47,XXX), 47,XYY, and triploidy account for more than 80% of significant chromosomal abnormalities diagnosed in the prenatal period [1]. Other chromosomal aberrations, such as balanced and unbalanced translocations, deletions, duplications, inversions, isochromosomes, and marker chromosomes account for only a small part of prenatally detected chromosomal abnormalities. Here, we present our experience of RAD by MLPA and aCGH in pregnancy with major congenital malformations.

Case reports

Case 1

A 26-year-old primigravid woman underwent cordocentesis at 25 gestational weeks because of congenital malformation of the fetal brain. Prenatal ultrasound at 25 gestational weeks revealed cebocephaly with a single nostril, semilobar holoprosencephaly (HPE), and tetralogy of Fallot (Fig. 1). About 2 mL blood was aspired, of which 1 mL of blood was applied for MLPA using SALSA MLPA P095 aneuploidy kit (MRC-Holland bv, Amsterdam, The Netherlands) to detect aneuploidies of chromosomes X, Y, 13, 18, and 21, and 1 mL of blood was applied for conventional cytogenetic analysis.

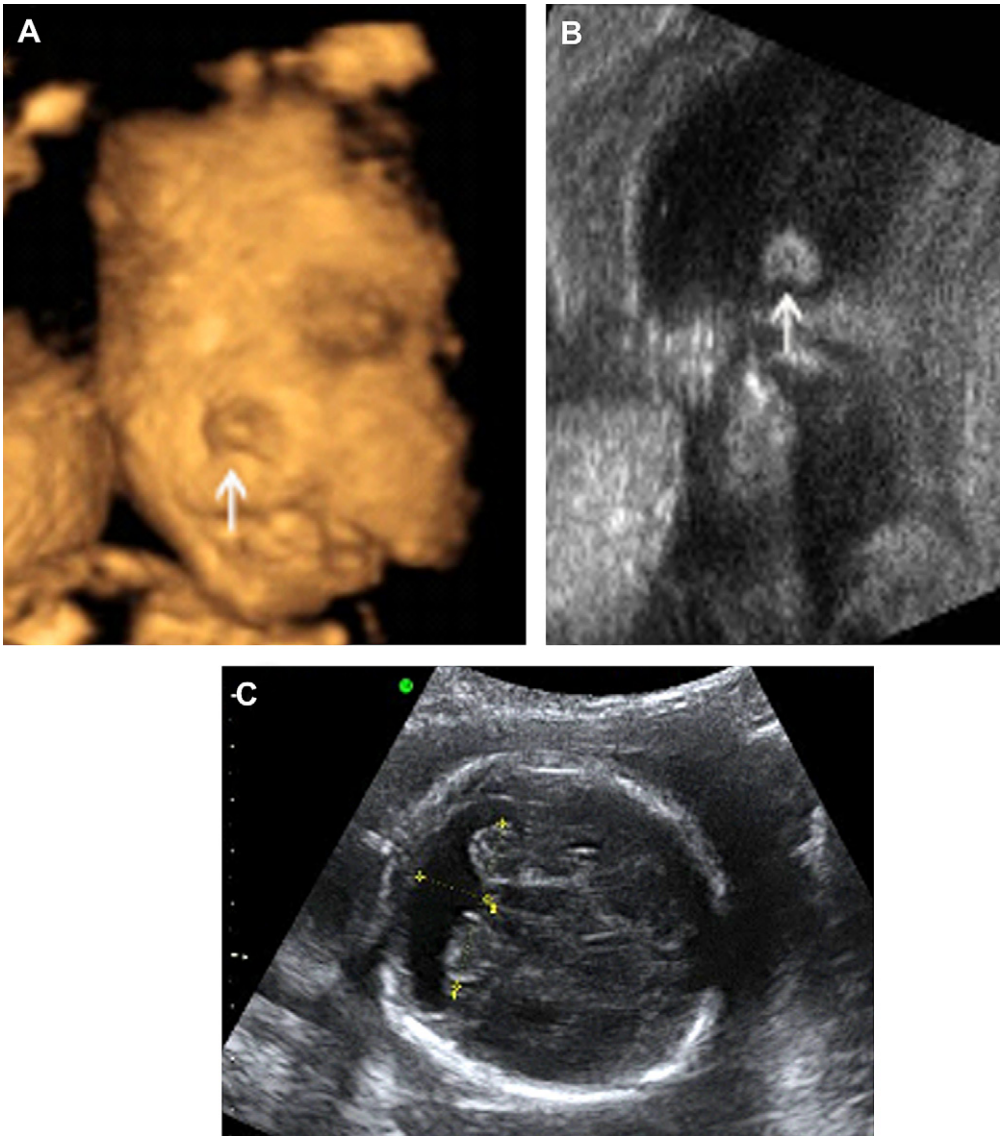

Fig. 1. Prenatal ultrasound of Case 1 at 25 gestational weeks shows cebocephaly with a single nostril (arrow) on (A) three-dimensional ultrasound, (B) two-dimensional ultrasound, and (C) semilobar holoprosencephaly.

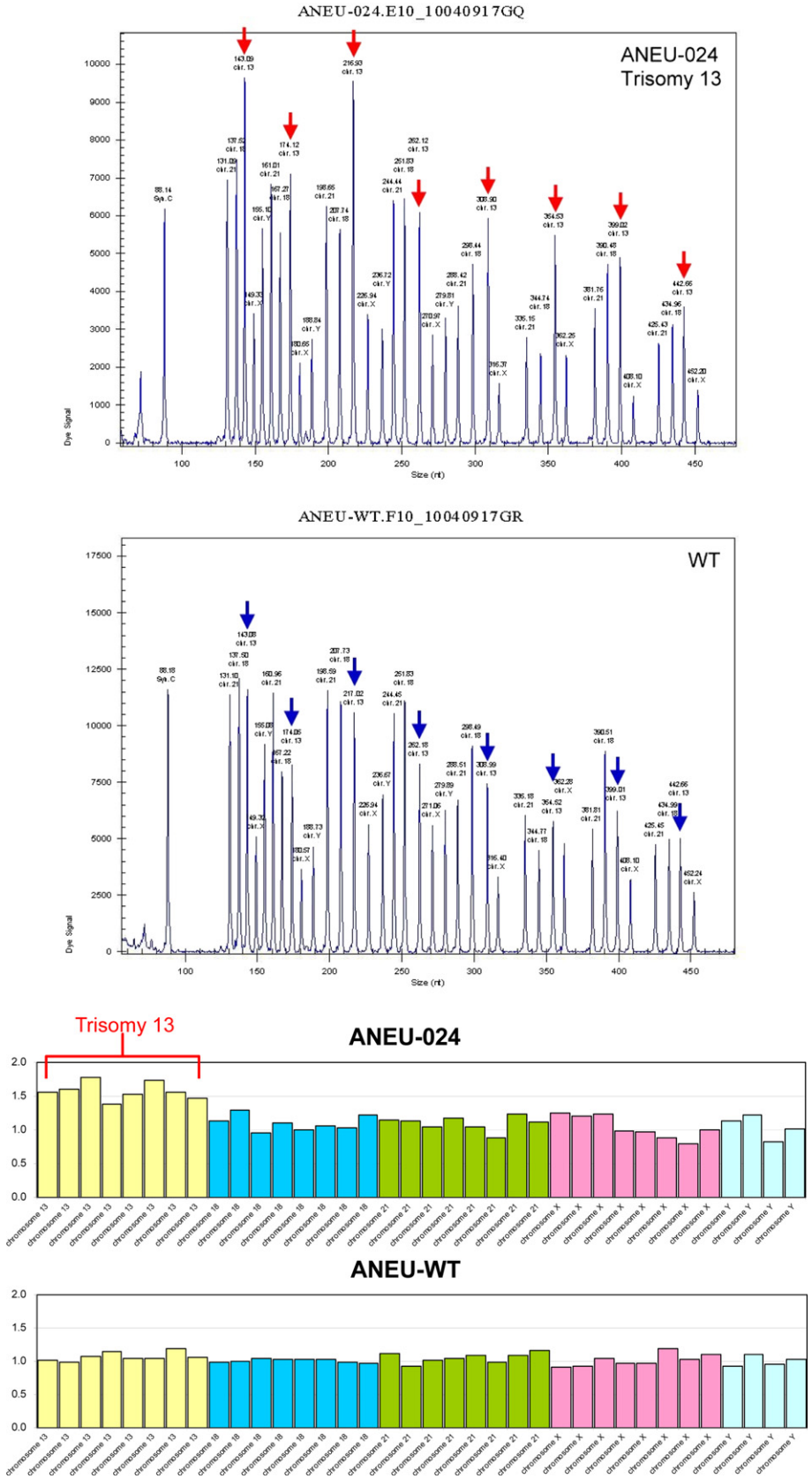

Fig. 2. Multiplex ligation-dependent probe amplification in Case 1 shows a male fetus with three copies of all targets on chromosome 13 consistent with the diagnosis of trisomy 13. The wild type in a normal male control has two copies of all targets on chromosome 13. Arrows indicate chromosome 13 targets. WT = wild type; ANEU = aneuploidy test; ANEU-024 = Case 1; ANEU-WT = wild type (control).

Within 2 days, MLPA showed the result of a male fetus with three copies of all targets on chromosome 13, two copies of all targets for chromosomes 18 and 21, and one copy of all targets for the X and Y chromosomes within the P095 kit [mlpa X,Y (P095) × 1, 13 (P095) × 3, 18,21 (P095) × 2] (Fig. 2), consistent with the diagnosis of trisomy 13. Conventional cytogenetic analysis revealed a karyotype of 47,XY,+13. The parents decided to terminate the pregnancy.

Case 2

A 39-year-old, gravida 3, para 2, woman underwent amniocentesis at 18 gestational weeks because of advanced maternal age. Prenatal ultrasound before amniocentesis revealed bilateral choroid plexus cysts, congenital diaphragmatic hernia (CDH), and club foot. The fetal biometry was equivalent to 17 weeks. About 32 mL amniotic fluid was aspired, of which 15 mL of amniotic fluid was applied for aCGH using uncultured amniocytes, and 15 mL was applied for conventional cytogenetic analysis using cultured amniocytes. Within 3 days, bacterial artificial chromosome (BAC)-based aCGH showed the result of trisomy 18 [arr cgh 18p11.32q23 (RP11-1150C18 → RP11-87C15) × 3] (Fig. 3). Eleven days after amniocentesis, conventional cytogenetic analysis revealed a karyotype of 47,XX,+18. The parents decided to continue the pregnancy. Prenatal ultrasound at 34 gestational weeks revealed polyhydramnios and a malformed fetus with intrauterine growth restriction, single umbilical artery, a flat facial profile, micrognathia, CDH, club feet, clenched hands, and a ventricular septal defect (Fig. 4).

Case 3

A 29-year-old, gravida 4, para 1, woman underwent amniocentesis at 23 gestational weeks because of fetal congenital anomalies. Prenatal ultrasound at 22 gestational weeks revealed aortic stenosis (AS) with a thickened aortic valve and a turbulent jet in the ascending aorta, and nuchal edema with a thick nuchal fold of 0.95 cm (Fig. 5). About 38 mL amniotic fluid was aspired, of which 20 mL of amniotic fluid was applied for aCGH using uncultured amniocytes, and 15 mL was applied for conventional cytogenetic analysis using cultured amniocytes. Within 3 days, BAC-based aCGH showed the result of monosomy X [arr cgh 1-22 (2,853 BAC) × 2, X (158 BAC) × 1, Y (27 BAC) × 0] (Fig. 6). Ten days after amniocentesis, conventional cytogenetic analysis revealed a karyotype of 45,X. The parents decided to terminate the pregnancy. The parental karyotypes were normal.

Case 4

A 37-year-old, gravida 5, para 3, woman underwent amniocentesis at 18 gestational weeks because of advanced maternal age and fetal congenital anomalies. Prenatal ultrasound at 17 gestational weeks revealed ventriculomegaly and nuchal cystic hygroma (Fig. 7). About 36 mL amniotic fluid was aspired, of which 20 mL of amniotic fluid was applied for aCGH using uncultured amniocytes, and 15 mL was applied for conventional cytogenetic analysis using cultured amniocytes. Within 3 days, BAC-based aCGH showed the result of trisomy 21 [arr cgh 21p11.2q22.3 (RP11-430M17 → RP11-1000I21) × 3] (Fig. 8).

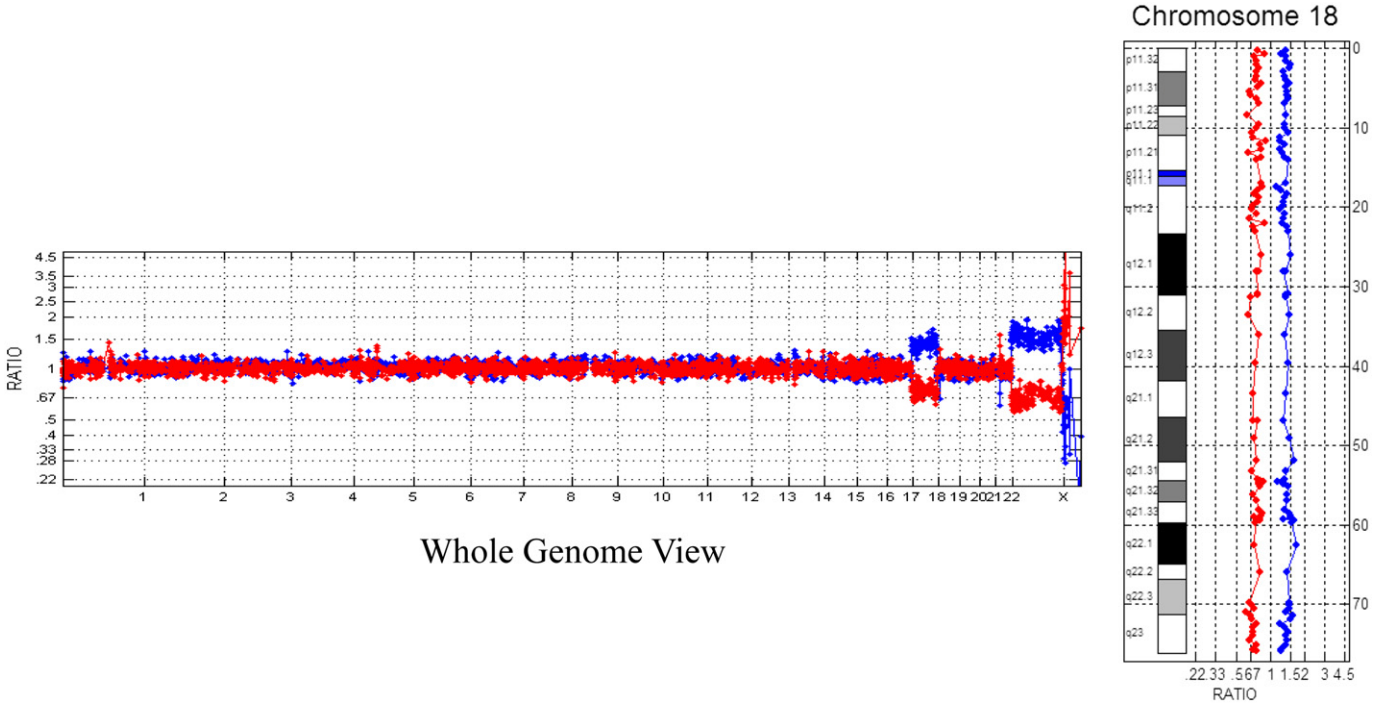

Fig. 3. Bacterial artificial chromosome–based array comparative genomic hybridization in Case 2 shows a duplication of chromosome 18 consistent with the diagnosis of trisomy 18.

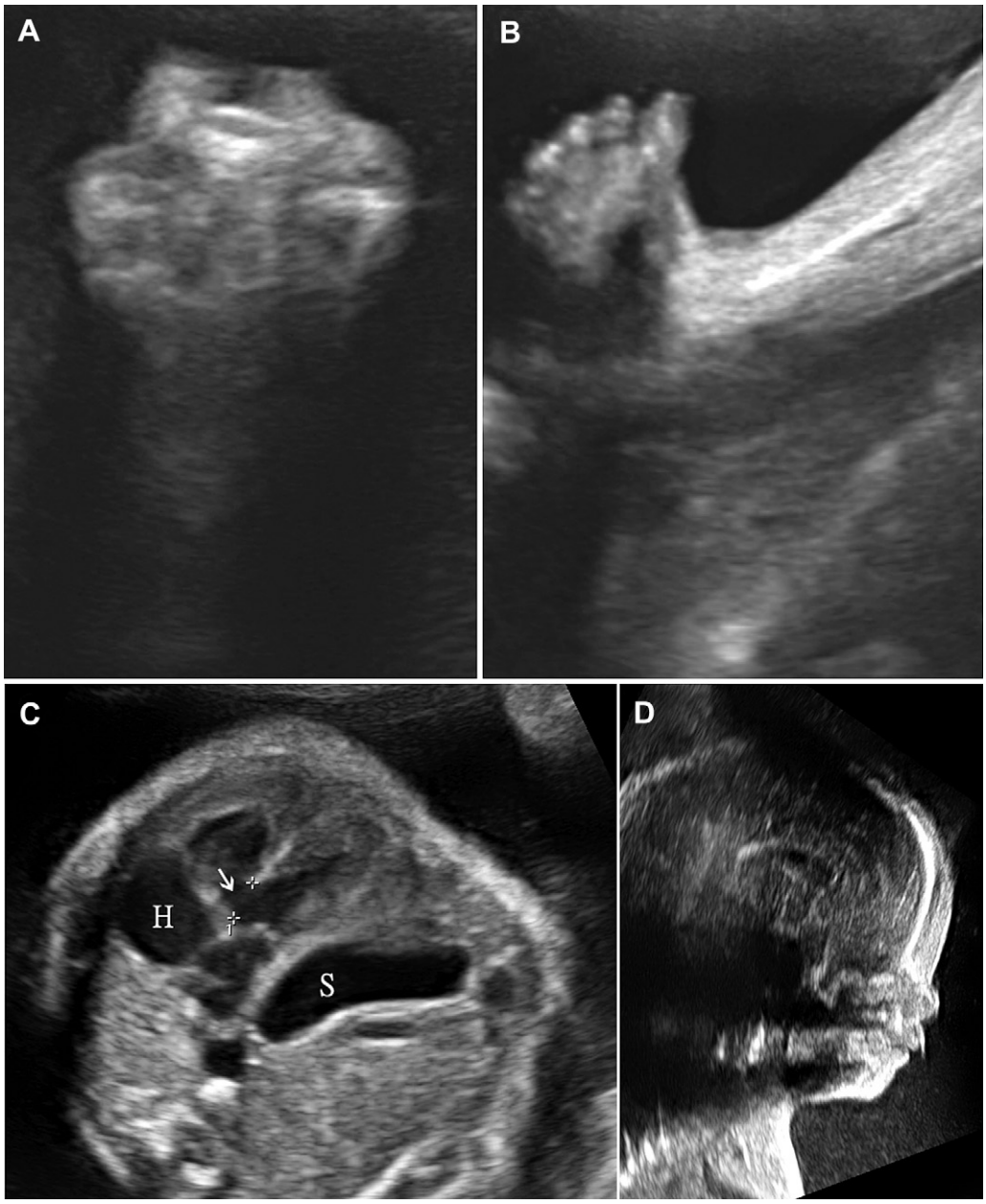

Fig. 4. Prenatal ultrasound of Case 2 at 34 gestational weeks shows (A) a clenched hand, (B) a club foot, (C) congenital diaphragmatic hernia with a stomach (S) in the chest and a heart (H) with a ventricular septal defect (arrow), and (D) a flat face with micrognathia.

Ten days after amniocentesis, conventional cytogenetic analysis revealed a karyotype of 47,XY,+21. The parents decided to terminate the pregnancy.

Case 5

A 37-year-old, gravida 2, para 1, woman underwent amniocentesis at 17 gestational weeks because of advanced maternal age and fetal congenital anomalies. Prenatal ultrasound at 17 gestational weeks revealed alobar HPE, mild hydronephrosis, and a small omphalocele containing the bowel (Fig. 9). About 38 mL amniotic fluid was aspired, of which 20 mL of amniotic fluid was applied for aCGH using uncultured amniocytes, and 17 mL was applied for conventional cytogenetic analysis using cultured amniocytes. Within 3 days, BAC-based aCGH showed the result of trisomy 13 [arr cgh 13q11q34 (RP11-631L24 → RP11-450H16) × 3] (Fig. 11). Ten days after amniocentesis, conventional cytogenetic analysis revealed a karyotype of 47,XY,+13. The pregnancy was subsequently terminated, and a 146-g fetus was delivered with premaxillary agenesis, omphalocele, and polydactyly of both feet (Fig. 10).

Discussion

MLPA, first described by Schouten et al [2], is a molecular method to detect gene dosage abnormalities in a wide range of conditions by relative quantification of up to 45 DNA target sequences in one polymerase chain reaction with the input of

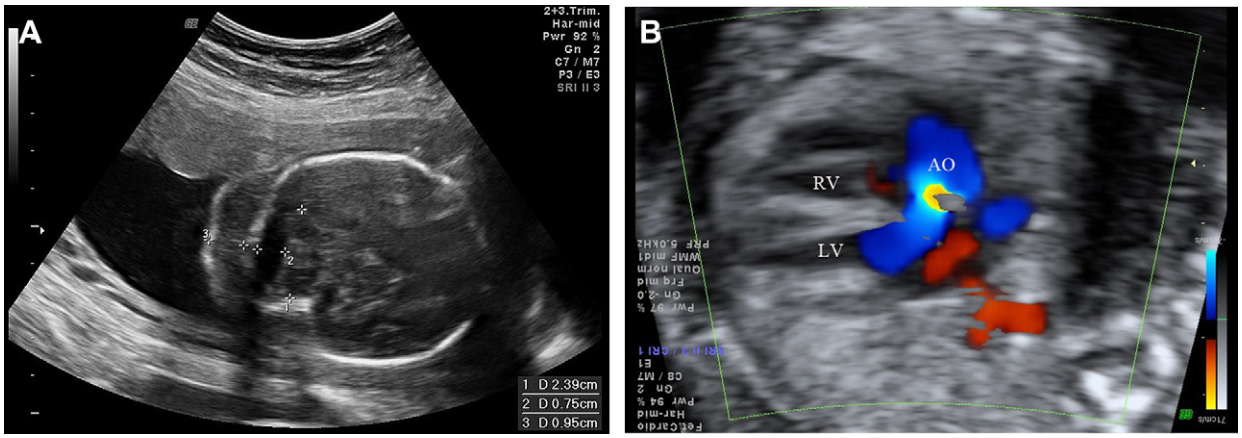

Fig. 5. Prenatal ultrasound of Case 3 at 22 gestational weeks shows (A) nuchal edema with a thick nuchal fold of 0.95 cm and (B) aortic stenosis with a thickened aortic valve and a turbulent jet (yellow color) in the ascending aorta. AO = ascending aorta; LV = left ventricle; RV = right ventricle.

20 ng or more DNA, but without the requirement of living cells or cell cultures. It can be automated and can obtain the result as quickly as 30 hours [3]. In MLPA, it is the probes added to the samples that are amplified and quantified [4]. The MLPA kit for RAD is commercially available. 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 the chromosome Y. MLPA can

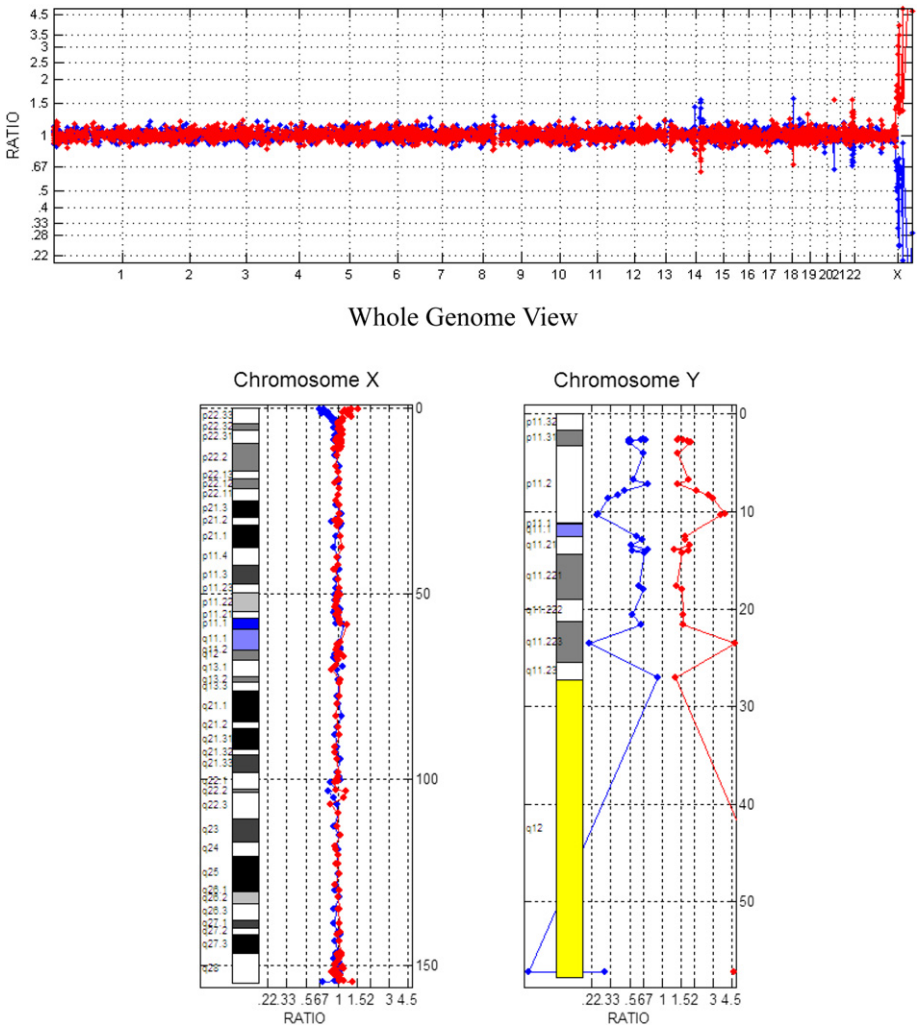

Fig. 6. Bacterial artificial chromosome–based array comparative genomic hybridization in Case 3 shows monosomy X consistent with the diagnosis of Turner syndrome.

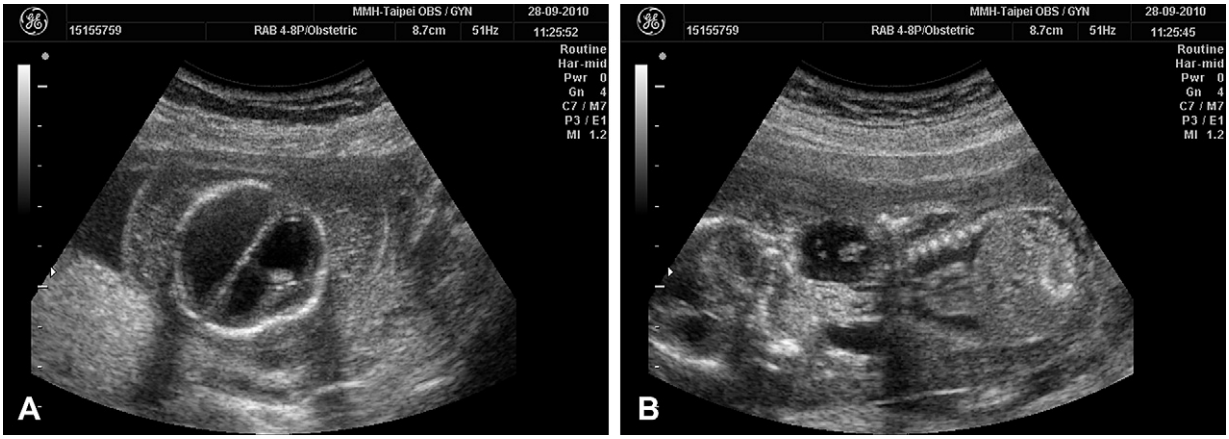

Fig. 7. Prenatal ultrasound of Case 4 at 17 gestational weeks shows (A) bilateral ventriculomegaly and (B) nuchal cystic hygroma.

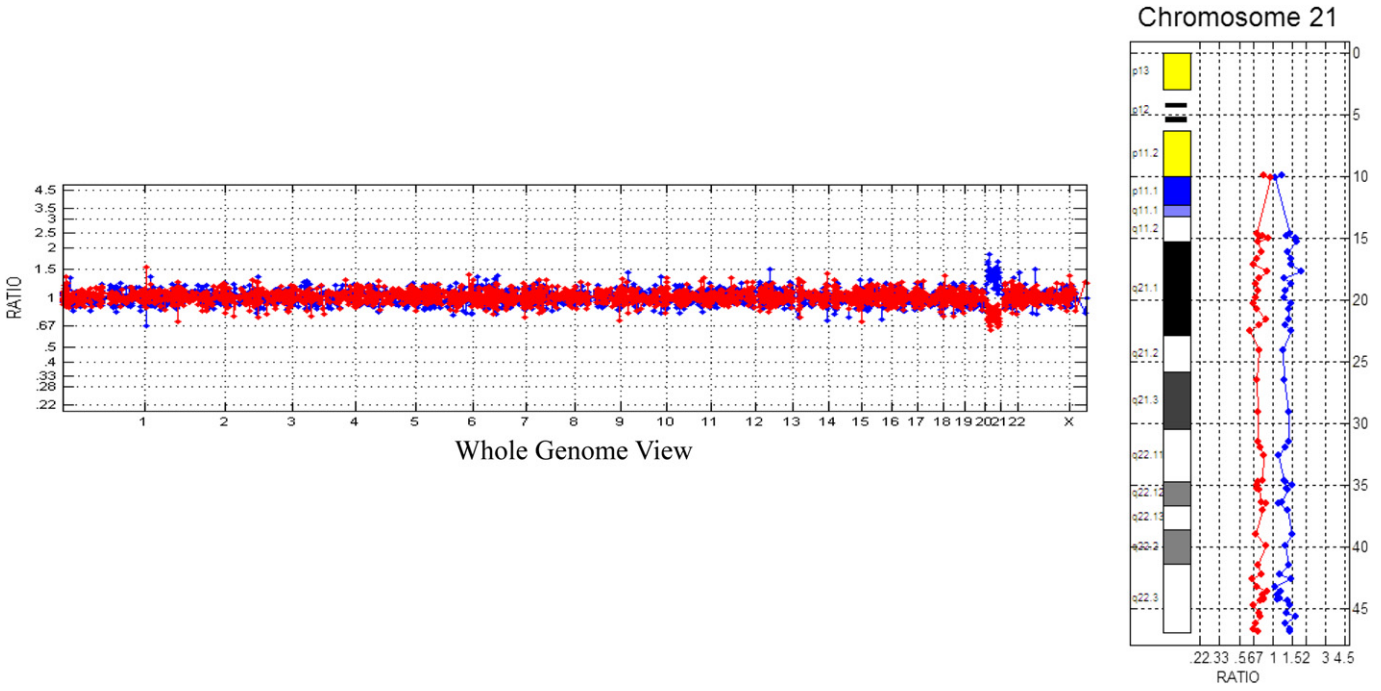

Fig. 8. Bacterial artificial chromosome–based array comparative genomic hybridization in Case 4 shows a duplication of chromosome 21 consistent with the diagnosis of trisomy 21.

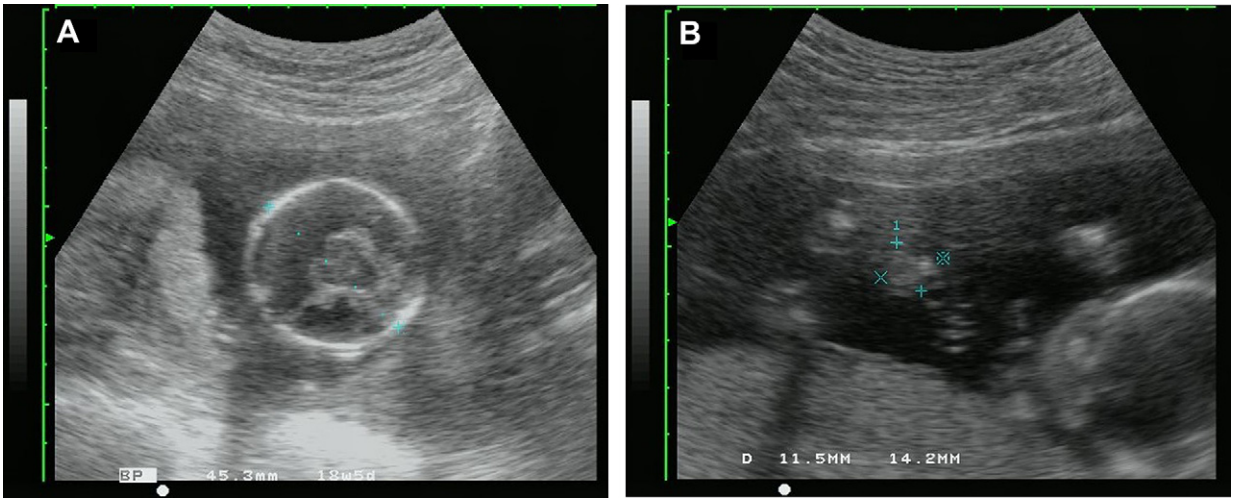

Fig. 9. Prenatal ultrasound of Case 5 at 17 gestational weeks shows (A) alobar holoprosencephaly and (B) a small omphalocele.

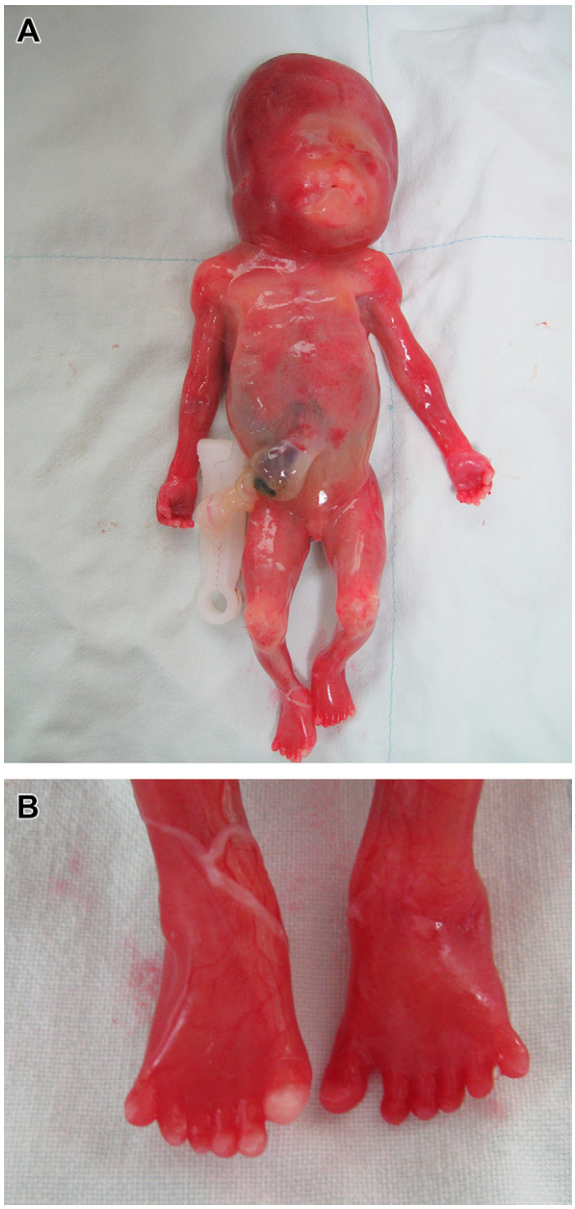

Fig. 10. (A) A trisomy 13 fetus with premaxillary agenesis, omphalocele, and polydactyly of feet. (B) Polydactyly of both feet.

reliably determine common aneuploidies. The diagnostic accuracy of MLPA for detecting common aneuploidies of chromosomes X, Y, 13, 18, and 21 is comparable with that of karyotyping [3,5–12]. Compared with conventional karyotyping, MLPA has the advantages of sparing the waiting time and reducing the cost, but the disadvantages of an inability to detect structural chromosomal abnormalities, maternal cell contamination, and triploidy [1,13]. It has been reported that MLPA is not expected to detect chromosome mosaicism [6,8]. However, in a prospective study of 4,000 amniotic fluid samples, Van Opstal et al [3] was able to detect some mosaic cases, structural chromosome aberrations, and male fetuses with 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.

BAC-based and oligonucleotide-based aCGH are comprehensive and high-resolution genome-wide screening methods to obtain DNA copy number information in a single rapid measurement without the requirement of living cells or cell cultures [1,13]. aCGH has the ability to detect DNA dosage imbalance including deletions and duplications and the inability to detect very low-level mosaicism, balanced translocations, inversions, and polyploidy. aCGH has proven to be invaluable for RAD of unbalanced chromosomal abnormalities in uncultured amniocytes [14]. Recent studies have suggested that aCGH can detect as little as 20% (and possibly 10%) mosaicism for the peripheral blood [15,16].

Our Case 1 was associated with HPE and cebocephaly. In this case, the facial dysmorphism of a single nostril was evident by two- and three-dimensional prenatal ultrasound. Chen et al [17] previously described the dysmorphism of a prominent nose and a large common nasal cavity in a trisomy 13 fetus with cebocephaly. Cytogenetic abnormalities have been reported in 24–45% of live births with HPE [18–20]. In a meta-analysis of chromosomal abnormalities associated with HPE, Blaas et al [21] suggested that the frequency was 34.7% (92/265) or more. Reported chromosomal abnormalities associated with HPE include trisomy 13, trisomy 18, triploidy, del(2p), dup(3p), del(7q), del(13q), del(18p), del(21q), and interstitial deletion of 14q13 [22,23]. Several new submicroscopic rearrangements have been identified by aCGH in HPE cases such as interstitial deletions of 1p, 6q, 10p, 16p, 18q, 20p, 21q, and Xp, and subtelomeric deletions in 19pter and 6qter [24]. HPE can be a first-trimester sonographic feature of fetuses in trisomy 13 pregnancies [25]. HPE has been found in 26.5% (48/181) of trisomy 13 fetuses in the first trimester [26]. In a meta-analysis of fetal trisomy 13 diagnosed in the second and third trimesters, Chen [25] found the mean frequency of HPE in trisomy 13 was 27% (64/237).

Our Case 2 was associated with CDH. Snijders et al [27] found that CDH was diagnosed in 10% of fetuses with trisomy 18. CDH can be associated with chromosomal abnormalities [28]. Trisomies 13, 18, and 21, and 45,X are the most common aneuploidies associated with CDH [29–31]. Frequently reported structural chromosomal abnormalities associated with CDH include tetrasomy 12p (Pallister-Killian syndrome), del(15)(q26.1–q26.2), del(8)(p23.1), cytogenetic rearrangements of 8q23, del(4)(p16), +der(22) t(11;22)(q23;q11), and del(1)(q41–q42.12) [30–33]. Other reported chromosomal rearrangements associated with CDH include duplication of 1q25–q31.2, deletion or duplication of 2q37, deletion of 3q22, deletion or duplication of 4q31, deletion of 5p15, deletion of 6p25, deletion of 6q25.3-qter, duplication of 8p21–p23.1, deletion of 8q22–q23, deletion of 9p24-pter, deletion of 11p13, duplication of 11q23.3-qter, duplication of 12p and duplication of 14q32 [31]. CDH has been seen in patients with tetrasomy 21, trisomy 22, and trisomy 9 [30,34,35].

Our Case 3 was associated with AS with nuchal edema. Snijders et al [27] found that nuchal edema was diagnosed in 6% of fetuses with Turner syndrome. About one-third of fetuses with nuchal edema have chromosomal abnormalities, mainly trisomies 21, 18, and 13 [28]. Song et al [36] found

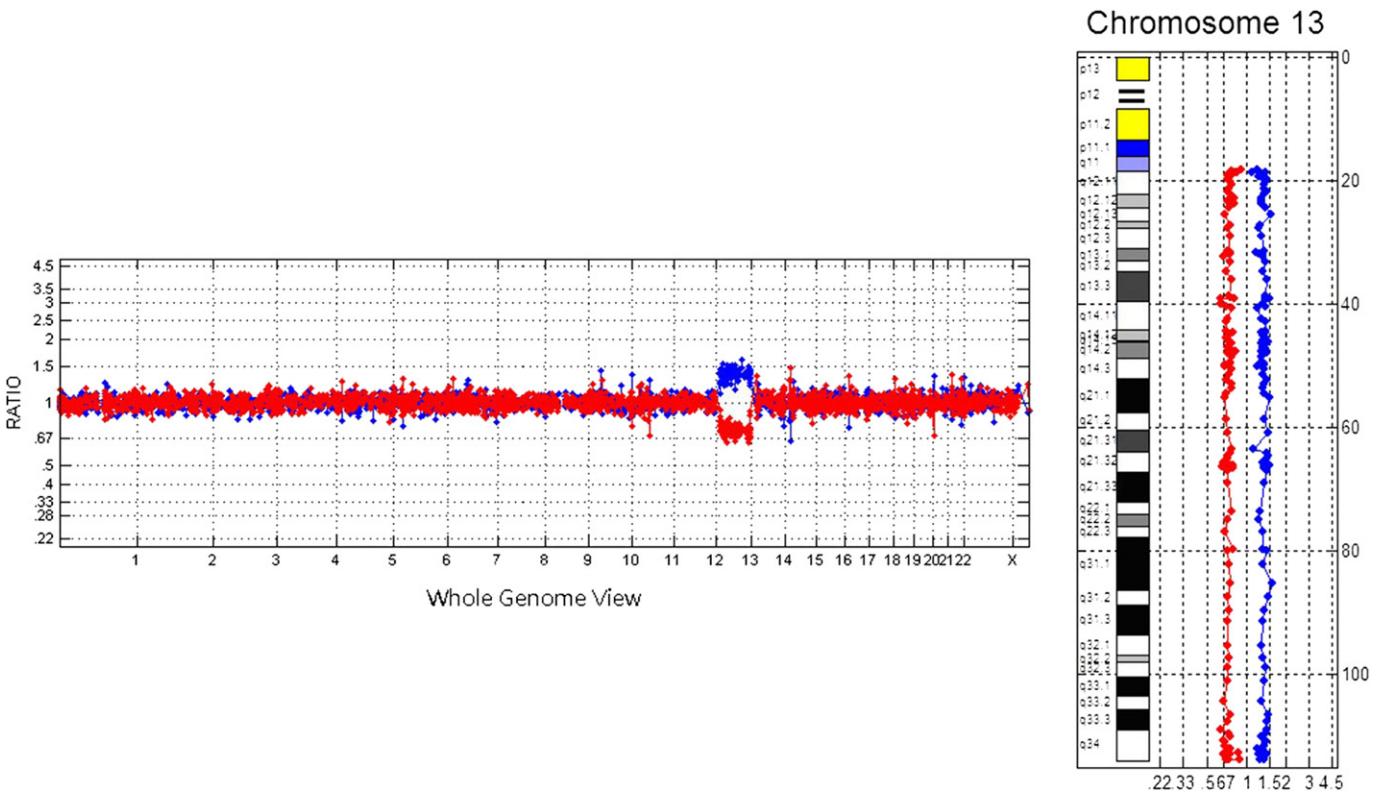

Fig. 11. Bacterial artificial chromosome-based array comparative genomic hybridization in Case 5 shows a duplication of chromosome 13 consistent with the diagnosis of trisomy 13.

chromosomal abnormality in 11.1% (1/9) of the fetuses with AS. In a study of 179 patients with Turner syndrome, Gøtzsche et al [37] found that 46 (25.7%) patients had cardiovascular malformations, 33 (18.4%) patients had aortic valve abnormalities, and 18 (10.1%) patients had aortic coarctation. Prandstraller et al [38] found that patients with Turner syndrome had a higher prevalence (29/136 = 21.3%) of congenital heart defects than the general population (21.3% *vs.* 0.8%, 27 times more frequent). Prandstraller et al [38] found that among the congenital heart defects, partial anomalous pulmonary venous return appeared to be the most prevalent anomaly (4/136 = 2.9%) (2.9% *vs.* 0.009%, 322 times more frequent) followed by aortic valve disease (AS and/or incompetence) (7/136 = 5.1%) (5.1% *vs.* 0.035%, 146 times more frequent) and aortic coarctation (6/136 = 4.4%) (4.4% *vs.* 0.043%, 102 times more frequent).

Our Case 4 was associated with ventriculomegaly and nuchal cystic hygroma. Snijders et al [27] found that ventriculomegaly was diagnosed in 16% of fetuses with trisomy 21. Ventriculomegaly can be associated with chromosomal abnormalities [23]. Snijders et al [27] reported chromosomal abnormalities in 13% of fetuses with prenatally detected ventriculomegaly, and trisomies 18, 13, and 21 and triploidy are the most common aneuploidies associated with ventriculomegaly. Snijders et al [27] found that cystic hygroma was diagnosed in 1% of fetuses with trisomy 21. Cystic hygroma can be associated with chromosomal abnormalities [28]. Snijders et al [27] reported chromosomal abnormalities in 68% of fetuses with prenatally detected cystic hygroma, and Turner syndrome, trisomy 21, and trisomy 18 are the most common aneuploidies associated with cystic hygroma.

Our Case 5 was associated with HPE, omphalocele, mild hydronephrosis, and polydactyly. Snijders et al [27] found that omphalocele was diagnosed in 17% of fetuses with trisomy 13. Snijders et al [27] reported chromosomal abnormalities in 35% of fetuses with prenatally detected omphalocele, and trisomies 18 and 13 are the most common aneuploidies. Snijders et al [27] found that mild hydronephrosis was diagnosed in 37% of fetuses with trisomy 13. The reported frequencies of polydactyly in fetuses with trisomy 13 range from 7.1% to 21.2% [28].

In conclusion, prenatal diagnosis of major congenital malformations should alert one to the possibility of chromosomal abnormalities. MLPA and aCGH have the advantage of RAD of common aneuploidies in cases with major congenital malformations.

Acknowledgments

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