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Review Article

Phenotype to genotype characterization by array-comparative genomic hydridization (a-CGH) in case of fetal malformations: A systematic review

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ABSTRACT

The aim of the current review is to report a-CGH abnormalities identified in fetuses with prenatally diagnosed fetal malformations in whom a normal karyotype was diagnosed with conventional cytogenetic analysis.

A systematic electronic search of databases (PubMed/Medline, EMBASE/SCOPUS) has been conducted from inception to May, 2017. Bibliographic analysis has been performed according to PRISMA statement for review. The following keywords were used: 'array-CGH' and 'fetal malformations" and "prenatal diagnosis"; alternatively, "microarray", "oligonucleotide array", "molecular biology", "antenatal diagnostics", "fetal diagnostics", "congenital malformations" and "ultrasound" were used to capture both "a-CGH" and "prenatal".

One-hundred and twelve fetuses with prenatally diagnosed fetal malformations with normal karyotyping and a-CGH abnormalities detected are described. Single or multiple microarray abnormalities diagnosed have been classified in relation to different organ/system affected. The most frequent a-CGH abnormalities were detected in cases of congenital heart diseases (CDHs), multiple malformations and central nervous system (CNS) malformations. Maternal or paternal carrier-state was seen in 19.64% (22/112), of cases while the number of reported *de novo* mutations accounted for 46.42% (52/112) of all CNVs microarray abnormalities. Array-comparative genomic hydridization (a-CGH) may become an integral and complemantary genetic testing when fetal malformations are detected prenatally in fetuses with normal cytogenetic karyotype. In addition, a-CGH enables the identification of CNVs and VOUS and improves the calculation of recurrent risk and the genetic counseling.

Introduction

The technical advancements of prenatal diagnosis of fetal malformations especially seen after the wide-spread introduction of 3D/4D ultrasound [1] together with molecular technology, have contributed to the genetic characterization of abnormal fetal phenotype. Studies have demonstrated that the use of a-CGH in case of fetal malformations over conventional fetal karyotyping carries an increased diagnostic rate of chromosomal imbalances [2–9]. A limitation of a-CGH is the potential to identify variants of unknown significance (VOUS), a finding seen in 3.4% of cases in the NICHD trial [10], requiring clarification.

It has been reported that a-CGH performed in fetuses with sonographic anomalies and normal karyotype detected clinically

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significant CNVs in 2% of cases [11]. Moreover, when rapid fetal karyotyping is clinically indicated, oligonucleotide a-CGH for direct analysis of uncultured amniocytes has shown to be feasible [12]. a-CGH is effective in screening for submicroscopic genomic imbalance in fetuses with *de novo* translocations, supernumerary marker chromosomes and add from 5.2% to 8.2% to the diagnostic field over conventional karyotyping in fetuses with abnormal ultrasound results [5].

This novel approach to the genetic understanding of fetal malformations by microarrays has direct implications: obstetricians involved in prenatal diagnosis are requested to gain an increased knowledge of the molecular mutations detected in order to request to geneticists the appropriate molecular searches, as conventional cytogenetic fetal karyotyping may not be anymore the standard of investigation [13]. Notwithstanding, a more close communication and collaboration between obstetricians and geneticists is needed and recommended in a modern prenatal diagnostic setting.

The primary aim of this review is to report a-CGH abnormalities detected in cases of prenatally diagnosed fetal malformations.

The secondary aim is to provide, both obstetricians and geneticists, with a clinical, useful roadmap when congenital anomalies are diagnosed during pregnancy, in order to facilitate phenotype-genotype characterization of congenital anomalies and improving the prenatal genetic counseling to parents by the multi-specialist team.

Materials and methods

Electronic searches of publications involving array-CGH abnormalities and their association with prenatally diagnosed phenotipical malformations in the fetus were performed. A comprehensive search of several databases (PubMed/Medline, EMBASE/SCOPUS) was conducted in any from each database from inception to May 2017, using MESH keywords and word variants for 'array-CGH' and 'fetal malformations" and "prenatal diagnosis". The following terms

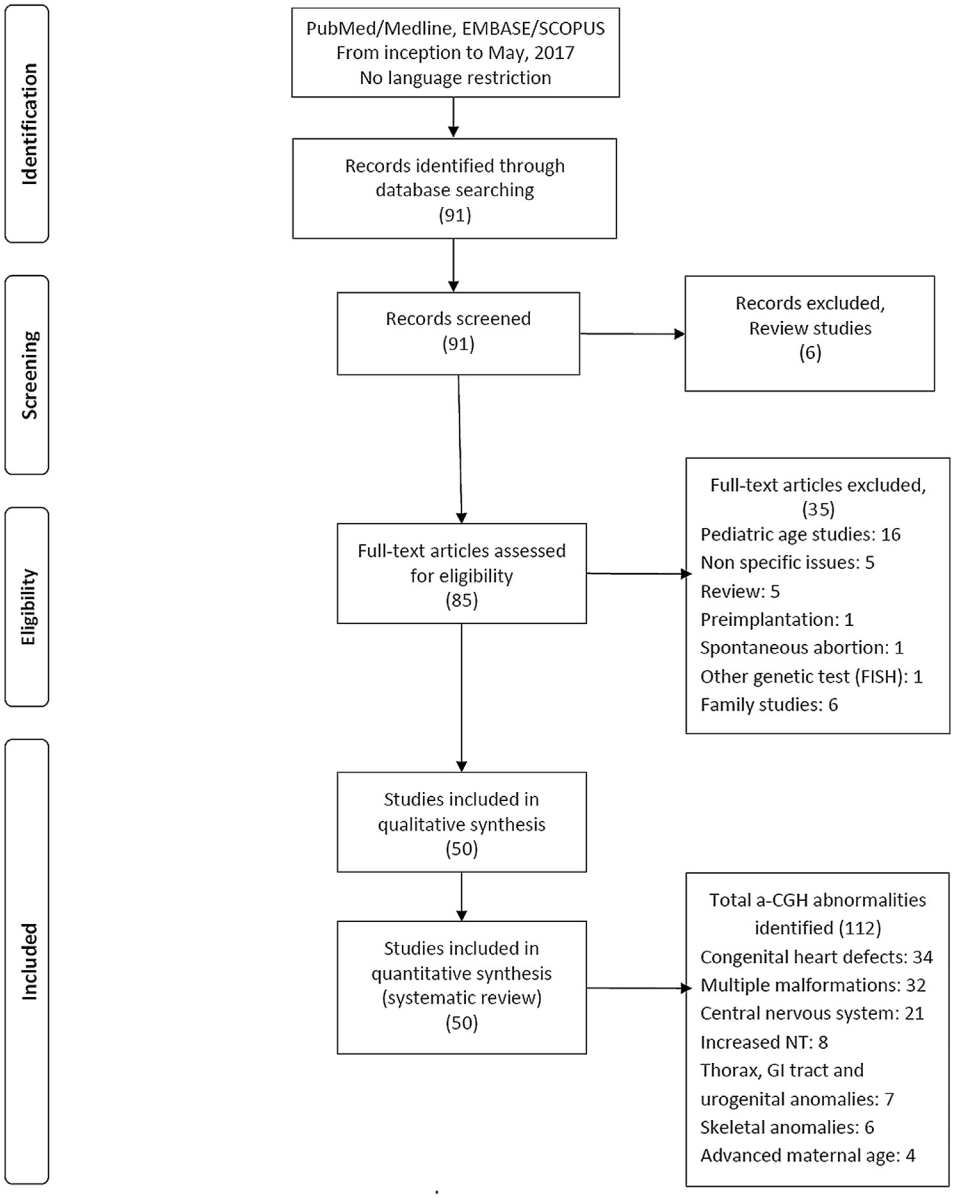

Fig. 1. Flowchart of electronic search of databases.

Table 1
Microarray results in cases of central nervous system (CNS), cranial, facial malformation with conventional karyotype and pregnancy outcomes in relation to maternal age (MA) and gestational age (GA) at diagnosis. (Legend: ACC: agenesis of the corpus callosum, Chr: chromosome; CL/CLP: cleft lip/cleft lip and palate, CPCs: choroid plexus cysts, CT-scan: computed tomography, MRI: magnetic resonance imaging, NR, not reported, SUA: single umbilical artery, PDA: patent ductus arteriosus, SHH, sonic hedge hoc; TOP: termination of pregnancy).

Ref. number (yr.)	Case	MA	GA	Karyotype	Microarray result (CNVs)	Gain or Loss size (Mb)	Pregnancy outcome
Ref. [15] (2015)	Case 1: Holoprosencephaly	NR	23w	Normal	arr 7q36 del (155,473,296 −158,909,738) × 1 encompassing the sonic hedgehog (SHH) gene	3.44 del at 7q36	TOP
Ref. [15] (2015)	Case 2: Hydrocephaly	NR	22w	Normal	arr 22q11.21 (18,648,855−21,800,471) × 3 dupl, including TBX1 gene	3.15 dupl at 22q11	TOP
Ref. [15] (2015)	Case 5: Holoprosencephaly, cleft lip and cleft palate (CL/CLP)	NR	23w	Normal	arr 2p21 del (44,749,075−45,098,283) × 1 which harbors SIX3 gene	0.34 del at 2p21	TOP
Ref. [15] (2015)	Case 6: Exencephaly	NR	21w	Normal	arr 19p12p13.11 del (19,838,485 −23,868,512) × 1	4.03 del at 19p12p13.11	TOP
Ref. [16] (2014)	Case G4: Holoprosencephaly, flat face and nasal bridge	NR	16w	45,XX,der(15)t(15;18) (q10;q10)	arr 18p11.32p11.21 *de novo*	14 dupl at 18p11	NR
Ref. [17] (2013)	Case 17: Bilateral ventriculomegaly	NR	NR	46,XY,del(1)(p36.3)dn	arr 1p36.33p36 .32(RP5-857k21-RP11-333E3) × 1 *de novo* 1p36 microdeletion syndrome	NR del at 1p36	TOP
Ref. [17] (2013)	Case 24: Agenesis of the corpus callosum (ACC) and meningocele	NR	NR	Normal	arr 5q35.3 (RP11- 281O15- > RP11-99H18) × 1 mat	del of 1.9 and dupl of 1.1	Vaginal delivery, admitted to SCBU, anomalies confirmed.
Ref. [18] (2013)	Microcephaly, ventriculomegaly, abnormal sulcal development with the absence of gyri and sulci and a shallow sylvian fissure. Fetal MRI confirmed corpus callosum dysgenesis and lissencephaly. Extracranial finding: single umbilical artery (SUA).	40yr	31w	46,XX del(17)(p13.3)	arr [hg19] 17p13.3 (0-3,165,530) × 1 mat (qPCR) (Miller-Dieker lissencephaly syndrome)	3.17 del at 17p13	Stillbirth at 32 weeks of gestation. Type of delivery: NR
Ref. [19] (2013)	Ventriculomegaly	NR	34w	46,XX,r(6)(p25;q27)	arr 6p25.3 del arr 6q26.27 del	1.3 del 6p25.3; 6.7 del 6q25-27	TOP at 38w
Ref. [20] (2012)	Case 1: Hypertelorism, epicanthic folds, depressed nasal bridge, long philtrum and low-set ears	35yr	17w	47,XX,r(2)(p11.1q21.2) [14]/ 46,XX; Interphase FISH revealed a mosaic level of 52%	Gain in chromosome 2 encompassing 2q11.2/q21.2; a small supernumerary marker chromosome derived from ring chromosome 2	39.4 dupl at 2q11.2/q21.2	TOP: GA not reported (NR)
Ref. [21] (2012)	Case 42: Lissencephaly	NR	NR	Normal	arr 17p13.3(RP11-629C16 → CTD-2386E6) × 1 *de novo*	2.1 del at 17p13	NR
Ref. [22] (2011)	Ventriculomegaly, oligohydramnios	NR	NR	Normal	arr 17q22-q23.1 (chr17:53,072,536 −56,612,662, hg18) including genes from MSI2 to BCAS3	3.54 del at 17q22-q23.1	TOP
Ref. [23] (2009)	Case 1: Holoprosencephaly	20yr	NR	46,XX FISH: unbalanced translocation t(7;8)	arr7q36.1q36.3(151,071,240−158,788,150) × 1 pat, 8q24.3(141,793,142−146,236,298) × 3 pat Partial monosomy 7q (deletion SHH); Partial trisomy 8q	del at 7.6 7q36.1q36.3; dupl at 4.4 of 8q24.3	NR
Ref. [23] (2009)	Case 5: Holoprosencephaly	22yr	NR	46,XX	arr 14q12q21.1(28,168,333−37,866,321) × 1 *de novo* interstitial del Partial monosomy 14q encompassing HPE8 region	9.7 del at 14q12.21.1	NR
Ref. [24] (2006)	Case 1: Borderline ventriculomegaly	38yr	18w	Interstitial deletion at the long arm of 1 chromosome 2	arr 2q14.2-2q21.3 *de novo* interstitial del	NR	At 5 months, the infant had febrile convulsions, atrophy of the left, undescended testis and developmental delay.

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Table 1 (*continued*)

Ref. number (yr.)	Case	MA	GA	Karyotype	Microarray result (CNVs)	Gain or Loss size (Mb)	Pregnancy outcome
Ref. [25] (2006)	Case 1: Holoprosencephaly	NR	NR	Unbalanced translocation of chromosomes 3 and chromosome 7	arr der(7)t(3;7)(q29;q36.3)	dupl of Chr.3; del of Chr. 4	NR
Ref. [26] (2003)	Case 1: Cerebral ventriculomegaly and bilateral choroid plexus cysts (CPCs)	24yr	24w	46,XY,-dup(1)(q32q44), FISH using whole chromosome paint (WCP) 1 showed uniform hybridization on the entire chromosome 1, including the distal half of chromosome 1q verifying a dupl of chromosome 1material. (Distal 1q chromosome syndrome)	arr 1q32q44q *de novo* dupl (1)	NR dupl at 1q32q44q	Birth at 37w, died at age 9 months. Postnatal phenotype: hypertelorism; short, downward-slanting palpebral fissures; high-arched and narrow palate. Feet overriding the second and third toes. Medium-sized patent ductus arteriosus (PDA). 11 pairs of ribs. CT-scan showed prominent posterior horns of the lateral cerebral ventricles
Ref. [27] (2002)	Severe retrognathia, rounded upper lips, glossoptosis, constant flexion of fingers of both hands	27yr	29w	46,XX,1q+	arr 1q23.1q31.1 interstitial dupl *de novo* (partial trisomy 1q)	NR dupl at 1q23	Preterm labour. Necropsy: Pierre Robin sequence and camptodactyly

were used to describe array comparative genomic hybridization: microarray, oligonucleotide array and molecular biology. Similarly, antenatal diagnostics, fetal diagnostics, congenital malformations" and "ultrasound" were used to capture 'prenatal'. Bibliographies of relevant articles were manually searched to identify papers not captured by the electronic searches. Disagreement of gray literature was resolved using Web of Science or by consensus (GT, EAJ). Studies addressing the role of genotype over phenotype in the fetus were initially selected. Cohort studies and clinical trials were included whilst reviews, studies based on preimplantation, abortions, use of different techniques (e.g. fluorescence in situ hybridization) and pediatric age/adult or family were excluded. Neither sample size nor languages was a criterion for exclusion. All data were reported on an Excel sheet. This form includes author's name, study setting, year of publication, type of the study, sample size, gestational age at diagnosis, ultrasound findings and/or fetal magnetic resonance imaging (f-MRI) findings, when present, age at delivery, pregnancy and perinatal outcome. We extracted data from studies using an extraction form that had been designed and pilot-tested by two authors (GT and EAJ). Attention was given in the assessment of the risk of biases that may affect the cumulative evidences. When the same case was reported in multiple publications, the main study report was used as the reference, and additional details were supplemented from the secondary report.

Results

A flowchart of the electronic searches, according to PRISMA guidelines [14], are displayed in Fig. 1.

Tables 1–7 [12,15–63] report genotype microarray abnormalities in relation to maternal age, gestational age at diagnosis, conventional fetal karyotyping, fetal malformations detected by ultrasound and/or fetal-MRI and pregnancy outcome. Abnormal fetal phenotype with specific-related a-CGH abnormalities have been classified according to the different anatomical district that was involved by structural malformation.

Overall, one hundred and twelve fetuses with congenital malformations detected prenatally, had had microarray assessment, following conventional cytogenetic karyotyping. A total number of 112 a-CGH abnormalities or copy number variations (CNVs) were identified of which 98 (87.50%) were single CNVs.

Microarrays abnormalities were most commonly detected in CHDs (30.35%, 34/112), followed by multiple congenital abnormalities (28.57%, 32/112) and CNS malformations (18.75%, 21/112). Currently, the lowest contribution of a-CGH to genetic characterization of congenital anomalies was observed in cases of advanced maternal age and skeletal dysplasia. In 88.23% of CHDs, only a single a-CGH was detected, with Di George (22q.11.2) syndrome as the most frequent microarray abnormality (55.88%); a single microarray abnormality was diagnosed in 90.62% of multiple congenital abnormalities and in 80.95% when CNS malformations were detected. Although skeletal dysplasias and advanced maternal age were the rearest malformations diagnosed prenatally, all cases were associated with a single CNVs microarray anomaly.

Overall, a maternal or paternal carrier-state was seen in 19.64% (22/112), of cases while the number of reported *de novo* mutations accounted for 46.42% (52/112) of all CNVs microarray abnormalities. The size of gain (microduplication) or loss (deletion) of targeted disease-specific regions of the genome detected by a-CGH were not reported in 19.64% of all microarray results.

Discussion

Nowadays chromosomal microarray (CMA), chromosomal genomic hybridization array (a-CGH) and single nucleotide

Table 2
Microarray results detected in cases of congenital heart defects (CHDs). (Legend: MA: maternal age, GA: gestational age, TOP: termination of pregnancy, NR: not reported, AoAC: aortic arch coarctation, AoA: aortic arch, AOT: abnormal outflow tract, ASD: atrial septal defect, AVSD: atrio-ventricular septal defect, CAVSD: complete atrioventricular septal defect, CHDs: congenital heart defects, CoA: coarctation of the aorta; DORV, double outlet of right ventricle, FGR: fetal growth restriction, HLHS: hypoplastic left heart syndrome, LPH: left pulmonary hypoplasia, IAoA: interrupted aortic arch, NA: not available, ND: not determined, PDA: patent ductus arteriosus, PE: pericardial effusion, PLSVC: persistent left superior vena cava, PRUV: persistent right umbilical vein, PS: pulmonary stenosis, PTA: persistent truncus arteriosus, RAoA: right aortic arch; TA: truncus arteriosus, TGA: transposition of great arteries, TOF: tetralogy of Fallot, VSD: ventricular septal defect).

Ref. number (yr.)	Case	MA	GA	Karyotype	Microarray result (CNVs)	Gain or Loss sie (Mb)	Pregnancy outcome
Ref. [28] (2016)	Case P171: Pulmonary stenosis (PS)	NR	NR	Normal	arr 15q11.2q13.1 (23,683,783 −28,453,340) × 1 del (Angelman/Prader–Willi syndrome)	4.77 del at 15q11	NR
Ref. [28] (2016)	Case P168: Ventriculo-septal defect (VSD)	NR	NR	Normal	arr 16p11.2 (29,567,295 −30,177,916) × 1 del	0.61 del at 16p11	NR
Ref. [28] (2016)	Case P398: Ventriculo-septal defect (VSD)	NR	NR	Normal	1q21.1q21.2 (146,043,713 −147,929,323) × 1 del	1.89 del at 1q21	NR
Ref. [28] (2016)	Case P025: Tetralogy of Fallot (TOF) with right aortic arch (RAoA)	NR	NR	Normal	arr 22q11.21 (Di George syndrome) del	3.16 del at 22q11	NR
Ref. [28] (2016)	Case P118: TOF	NR	NR	Normal	arr 22q11.21 del	1.81 del at 22q11	NR
Ref. [28] (2016)	Case P235: Double outlet right ventricle (DORV)	NR	NR	Normal	arr 22q11.21 del	2.82 del at 22q11	NR
Ref. [29] (2016)	TOF	NR	25w 3 d	46,XY, inv(9)(p11q13),mat	arr[hg19] 9q34.13q34.3(135,550,093 −141,018,648) × 3 dupl *de novo*; 16p11.2(32,564,735 −33,814,547) × 1 del	Gain 5.4 at 9q34; 1.25 del at 16p11	TOP at 32w. Necropsy: TOF, small jaw, flat nasal bridge, high-arched palate
Ref. [30] (2015)	Case 1: Transverse AoA hypoplasia Mother: 46,XX, t(8;16)(p21;q24)	NR	NR	NR	46,XX,der(16)t(8;16)(p21;q24) mat Trisomy 8p23.3-p21.3	NR	NR
Ref. [31] (2015)	Case 1: Ventricular septal defect (VSD)	NR	NR	NR	arr[hg19] 22q11.21q11.22(19,172,842 −22,691,548) × 1 *de novo*	3.5 del at 22q11	NR
Ref. [31] (2015)	Case 4: Congenital heart defect (CHD)	NR	NR	NR	arr[hg19] 22q11.21(19,172,841 – 19,843,647) × 1	670 kb del at 22q11	NR
Ref. [31] (2015)	Case 5: RAoA with right ductus arteriosus (DA)	NR	NR		arr[hg19] 22q11.21(19,172,842 – 19,843,647) × 1	670-kb del at 22q11	NR
Ref. [31] (2015)	Case 12: Pericardial effusion, mild PS, right ventricular hypertrophy	NR	NR	NR	arr[hg19]7q11.23(72,583,172–74,227,094) × 1 del (Williams-Beuren syndrome)	1.6 del at 7q11	NR
Ref. [32] (2014)	TOF VSD	NR	21w	Normal	arr 22q11.21(19,411,059 −19,835,557) × pat	NR del at 22q11	NR
Ref. [32] (2014)	Coarctation of the aorta (CoA)	NR	23w	Normal	arr19q13.42q13.43(59,640,794 −61,566,588) × 3 *de novo*	NR	NR
Ref. [32] (2014)	VSD	NR	17w	Normal	arr 16p13.11(14,817,705 −16,679,871) × 1 mat	NR	NR
Ref. [32] (2014)	Abnormal outflow tract (AOT), hypoplastic left heart syndrome (HLHS), situs inversus	NR	34w	Normal	arr16p13.11p12.3(15,311,952 −18,539,483) × 1 *de novo*	NR	NR

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Table 2 (*continued*)

Ref. number (yr.)	Case	MA	GA	Karyotype	Microarray result (CNVs)	Gain or Loss sie (Mb)	Pregnancy outcome
Ref. [32] (2014)	AOT	NR	19w	Normal	arr8q12.1(61,104,853 −62,025,350) × 1 *de novo*	NR	NR
Ref. [32] (2014)	AOT	NR	21w	Normal	arr8p23.1(11,578,132 −11,789,207) × 1 *de novo*	NR	NR
Ref. [32] (2014)	Hypoplastic right heart syndrome (HRHS)	NR	18w	Normal	arr 8q11.1(46,966,687 −47,976,420) × 3 pat VOUS	NR	NR
Ref. [33] (2014)	Case 1: VSD, PAS	NR	24w	Normal	arr 17p13.3(87,009 −1,184,534) × 1; 22q11.21(18,919,942 −21,440,514) × 1	1.10 del at 17p13; 2.52 del at 22q11	TOP with necropsy confirmation
Ref. [33] (2014)	Case 2: DORV, CoA/interrupted AoA, mitral atresia, PLSVC, VSD, coronary sinus dilatation	NR	26w 4 d	Normal	arr 8p23.1	4.09 dupl at 8p23.1	TOP with necropsy confirmation
Ref. [33] (2014)	Case 3: TOF	NR	23w2d	Normal	arr10q26.3(131,585,685 −134,832,720) × 1 10q26.3 syndrome	3.25 del	TOP. Necropsy: VSD, overriding aorta, right ventricular hypertrophy
Ref. [34] (2014)	Overriding aorta, small pulmonary artery, VSD	NR	26w	Normal	arr 22q11.2 del encompassing the DGCR6, DGCR2, DGCR14, UFD1L, TBX1, GNB1L, COMT, DGCR8, DGCR6L, and MED15 genes	3.07 del at 22q11	Fetus had prenatal hypertelorism, prominent nasal root, bulbous nasal tip, micrognathia, low-set ears
Ref. [35] (2014)	Case 1: CoA, VSD, right hand preaxial hexadactyly	37yr	26w	Normal	arr 8p23.1 dupl *de novo*	1.43 dupl at 8p23	TOP at 28w with necropsy confirmation
Ref. [36] (2014)	Case 3: TOF	NR	NR	Normal	arr 22q11.21 (19,746,363 −19,747,209) × 1 *de novo* encompassing TBX1 gene	0.85 kb del at 22q11	Necropsy: TOF confirmed.
Ref. [36] (2014)	Case 7: Interrupted AoA	NR	NR	Normal	arr 16p11.2 (32,624,578 −33,604,468) × 3 pat dupl encompassing 19 genes	979.89 kb dupl at 16p11 encompassing 15 genes	Necropsy: not available (NA)
Ref. [36] (2014)	Case 8: TGA, VSD	NR	NR	Normal	arr 22q11.21 (19,746,363 −19,754,877) × 3 dupl *de novo* encompassing TBX1 gene	8.51 kb dupl at 22q11	Necropsy: TGA, VSD confirmation.
Ref. [37] (2013)	Case 1516: TOF	NR	20w4d	ND	arr 16p13.12p13.11 *de novo*	1.5 del at 16p13	NR
Ref. [37] (2013)	Case 982: TOF	NR	18w6d	46,XX	arr 11p15.5 (210,100–8,190,071)×3 17p13.3 (86,809–1724,253) x 1 *de novo*	7.9 dupl	NR
Ref. [17] (2013) Hillman	Case 1: Truncus arteriosus (TA)	NR	NR	Normal	arr Xp22.32p22.31(RP11-60N3- > RP11-769N24) ×2, Xp22.3 (RP11-44F2) × 2 mat (possible disruption NLGN4VOUS)	NR	Postnatal cardiac surgery; normal development at 7 months
Ref. [17] (2013)	Case 19: CoA, VSD	NR	NR	Normal	arr 22q11.2(RP11-800B02- > RP11-330P17) × 1 *de novo*	NR	Miscarriage
Ref. [17] (2013)	Case 20: DORV	NR	NR	Normal	arr 22q11.21(RP11-800B02- > RP11-330P17) × 1 *de novo*	NR	TOP

Ref. [17] (2013)	Case 21: TOF	NR	NR	Normal	arr 22q11.21(RP11-800B02-> RP11-330P17) × 1 *de novo*	NR	TOP
Ref. [17] (2013)	Case 22: TA	NR	NR	Normal	arr 22q11.21(RP11-800B02-> RP11-330P17) × 1 mat	NR	TOP with necropsy confirmation of TA type 1
Ref. [38] (2013)	Left−right axis malformations, TGA, VSD	38yr	NR	46,XY	arr 9q33.1q33.3 del	6.97 del at 9p33	NR
Ref. [39] (2013)	AoA coarctation, left pulmonary hypoplasia (LPH). Family history of 2 previous male foetuses with partial trisomy 18p, partial monosomy 18q and AoA coarctation	NR	38w	46,XY, der (18) (pter→q21.3::p11.2-pter)	arr 18q21.33→qter del *de novo*; 18pter→p11.21 dupl *de novo*	17.7 del at 18q21qter; 12.4 dupl at 18pter→p11.21	Delivered at 38w5d. Low-set ears. Died 2 days of life
Ref. [21] (2012)	Case 38: TOF	NR	NR	47,XY,+der(14) t(3;14)(p24.1;q21.1) mat	arr 3p26.3p233p26.3p23(CTC-228K22→RP11-1097L4) × 3	26.61 dupl at 3p; 18.94 dupl at 14q	TOP
Ref. [21] (2012)	Case 37: PAS, VSD	NR	NR	46,XX	arr 22q11.21(RP11-690P21→RP11-1116M14) × 1 del *de novo*	2.4 del at 22q11	TOP
Ref. [21] (2012)	Case 34: TOF Case 35: TOF Case 36: TOF	NR	NR	46,XYl 46,XY 46,XYl	arr 22q11.21(RP11-690P21→RP11-1116M14) × 1 del *de novo* arr 22q11.21(RP11-690P21→RP11-1116M14) × 1 del *de novo* arr 22q11.21(RP11-690P21→RP11-1116M14) × 1 del *de novo*	2.4 del at 22q11 2.4 del at 22q11 2.4 del at 22q11	TOP TOP TOP
Ref. [21] (2012)	Case 33: TOF	NR	NR	46,XX	arr 4q35.2(RP11-213A19→RP11-521G19) × 1 mat	3.61 del at 4q35	TOP

Table 3
Microarray results in cases of skeletal malformations.

Ref. number (yr.)	Case	MA	GA	Karyotype	Microarray result (CNVs)	Gain or Loss size (Mb)	Pregnancy outcome
Ref. [40] (2013)	Case 2: Polydactyly	36yr	NR	NR	arr Yq11.223q11.23 del	3.0 del at Yq11	Term birth
Ref. [40] (2013)	Case 14: Single forearm bone, hyperflexed hand, agenesis of the left leg	40yr	NR	NR	arr 19q13.2-q13.31 del	0.5 del at 19q13.2	TOP
Ref. [37] (2013)	Case 769: Micromelia	NR	15w 3 d	Normal	arr 11q22.3 (103,141,484 −103,306,930) x 1 pat	0.17 del at 11q22.3	NR
Ref. [37] (2013)	Case 1484: Arthrogryposis multiplex congenital (AMC)	NR	18w 2 d	Normal	arr[hg19] 22q11.21(18,894,835 −21,464,119) x 1 del	2.6 del at 22q11.2	NR
Ref. [21] (2012)	Case 44: Polydactyly, camptodactyly, hypoplasia of the fifth finger	NR	NR	46,XY	arr 8q24.13q24.21(RP11-689C11- > RP11-440N18) x1 *de novo*	5.69 del at 8q24	NR
Ref. [41] (2012)	Short femur	34yr	22w	46,XY,del(15)(q21;q21)	46,XY,del(15)(q15.3q21.3)	11.1 del	TOP at 36w. Necropsy: prominent forehead, bow-shaped eyebrows, down slanting palpebral fissures, low-set and small ears, depressed nasal bridge, convex nasal ridge, under-developed long philtrum, a thin upper vermillion, a mouth held open, micro-retrognathia and bilateral megaurethra

Table 4
Microarray results in cases of thorax, gastro-intestinal tract (GIT) and urogenital malformations. (Legend: CDH: Congenital diaphragmatic hernia, C-s: Cesarean section, HNPP: hereditary neuropathy with liability to pressure palsies; LUTO: lower urinary tract obstruction, MCDKD: multicystic dysplastic kidney disease).

Ref. number (yr.)	Case	MA	GA	Karyotype	Microarray result (CNVs)	Gain or Loss size (Mb)	Pregnancy outcome
Ref. [16] (2014)	Case G5: Congenital diaphragmatic hernia (CDH)	NR	30w	46,XY,del(14)(q24.2;q32.11)	arr 14q24.2q32.11 del *de novo*	17 del	NR
Ref. [37] (2013)	Case 1272: Multicystic dysplastic kidney disease (MCDKD)	NR	14w2d	Normal	arr[hg19] 7p13p12.3(45,060,426 −47,393,195) x 1 *de novo*	2.2 del at 7p13p12.3	NR
Ref. [21] (2012)	Case 48: Exomphalos	NR	NR	46,XY	arr 7p22.3p22.1(RP11-90P13- > RP11-936A1) × 1, 9p24.3p24.3(RP11-1112G24 - > RP11-635C16) × 3 *de novo*	5.62 del at 7p22; 2.19 dupl at 9p24.3	NR
Ref. [17] (2013)	Case 23: Bladder outflow obstruction (LUTO)	NR	NR	Normal	arr 17p12(RP1-27J12-RP11-385D13) x 1 pat, include gene PMP22: hereditary neuropathy with liability to pressure palsies (HNPP)	NR	TOP before result of microarray available. Necropsy: NA.
Ref. [42] (2013)	Case 1: Bilateral hydronephrosis, ↑ echogenicity of both kidneys	35yr	18w	46,XY	arr [hg19] 17q12 (34,653,178 −36,402,867) × 1 del involving 15 OMIM genes including LHX1 and HNF1B. Whole-genome aCGH analysis on the DNA extracted from maternal blood detected a 1.54-Mb del at 17q12, or arr [hg19] 17q12 (34,814,526e36,355,604) × 1	1.75 del at 17q12	TOP at 23 weeks. A 737-g male fetus was delivered with no facial dysmorphism and abnormalities of male external genitalia.
Ref. [43] (2012)	Case 53: Hyperechogenic kidneys, bilateral hydronephrosis, vesical distension, urethral cyst, mega rettum	NR	NR	46,XY	arr10q26.13q26.3(124,590,071-132,729,781) × 1 del *de novo*	8.14 del at 10q26	TOP. Autopsy: Potter's facies, obstructive MCDKD, dilated sigmoid, mega rectum, imperforated anus
Ref. [44] (2015)	CDH	NR	19w	46,XX The presence of mosaicism for isochromosome 12p was sought and Pallister-Killian syndrome excluded using FISH	arr 17q12 del causing haploinsufficiency for 17 genes, including AATF, ACACA, DDX52, DUSP14, GGNBP2, HNF−1B, LHX1, PIGW, SYNRG, TADA2A, and ZNHIT3.	1.4 de at 17q12	Preterm labour at 34w. Repeat C-s: female infant of 1600 g. Died 2 h later. Necropsy: NP.

Table 5
Microarray results in cases of multiple congenital abnormalities (MCA). (Legend: AMC: arthrogryposis multiplex congenital, ASD: atrial septal defect; DM: diabetes mellitus; DWS: Dandy Walker Syndrome, DWV: Dandy Walker variant, IUFD: intrauterine fetal death, MSAoA: mild stenosis of aortic arch; PRUV: persistent right umbilical vein; SCyH: septated cystic hygroma; sSMCs: small supernumerary marker chromosomes).

Ref. number (yr.)	Case	MA	GA	Karyotype	Microarray result (CNVs)	Size (Mb)	Pregnancy outcome
Ref. [15] (2015) Sun	Case 3: Dandy Walker Syndrome (DWS) + VSD and PLSVC	NR	25 + w	NR	arr 2q13q14.1(111,596,906 −21,800,471) × 3 del encompassing 4 OMIM genes: PAX8, IL1B, MERTK, IL1RN associated with CHDs, facial and fingers malformations and neurodevelopmetal impairement	3.25 del at 2q13	TOP. Necropsy confirmation.
Ref. [15] (2015)	Case 4: DWS + skeletal dysplasia + CL/CLP, agenesis of septum pellucidum + arachnoid cyst	NR	23 + w	Male fetus	arr Xq13.3(74,171,888.114,844,660) del	0.17 del at Xq	TOP. Necropsy confirmation.
Ref. [15] (2014)	Case 5: Holoprosencephaly, CL/CLP	NR	23 + w	NR	arr 2p21(44,749,075−45,098,283) × 1 del encompassing SIX3 gene	0.34 del at 2p21	TOP. Necropsy confirmation.
Ref. [15] (2015)	Case 7: Holoprosencephaly + facial anomalies + VSD	NR	24 + w	NR	arr 4q35.2(187,079,723−190,767,114) × 1 del encompassing OMIM gene FAT1.	2.79 del at 4q35.2	TOP. Necropsy confirmation.
Ref. [15] (2015)	Case 8: Hydrocephaly, ↑ nuchal fold, FGR, sacrococcygeal vertebral anomaly	NR	25 + w	NR	arr 21q21.1 del *de novo* encompassing the NCAM2 gene; CMA in both parents was negative	1.15 del at 21q21	TOP. Necropsy confrimation.
Ref. [31] (2015)	Case 7: TOF and absent stomach	NR	22 + w	NR	arr[hg19] 6q16.1(93,609,026 −96,533,581) × 1 del compassing 4 OMIM genes. Lymphedema-distichiasis syndrome with renal disease and DM.	880 kb del at 6q16.1	TOP. Necropsy: VSD, overriding aorta, RV hypertrophy
Ref. [31] (2015)	Case 9: Hypoplastic nasal bone, echogenic bowel	NR	NR	NR	arr[hg] 1q21.1q21.2(145,373,269 −147,780,608) × 3 mat 1q21.1 dupl syndrome encompassing 22 OMIM genes	2.4 dupl at 1q21	NR
Ref. [31] (2015)	Case 11: Previous pregnancy with Beckwith−Wiedemann syndrome	39yr	NR	NR	arr 11p15.5(2,337,102−2,763,614) × 4 pat triplication encompassing 6 OMIM genes (Beckwith−Wiedemann syndrome)	426-kb tripl. at 11q15	NR
Ref. [45] (2015)	↑ NT, exomphalos. At 16w- 18w: micrognathia, TOF	37yr	20w	46,XY	arr 15q11.2 dupl (15q11.2(20,220,446 −20,629,325) × 3 pat	408 kb dupl at 15q11	TOP at 20w. Necropsy confirmation.
Ref. [46] (2014)	Polyhydramnios, FGR, PRUV, MSAoA	NR	18w	46,XX	arr 11q22.3→q23.3(107,686,511 −116,660,613) × 1 del *de novo* encompassing FRA11B and FRA11G.	8.97 del at 11q22	TOP at 30w: no apparent phenotype abnormalities Necropsy refused by parents-
Ref. [47] (2014)	Case 1: Bilateral CPCs, SUA, oesophageal atresia	NR	20w	46,XX with a small non-mosaic interstitial deletion of chromosome 2q33q35	arr 2q33.2q35(204,394,564 −219,189,331) segmental monosomy del *de novo* encompassing 52 OMIM genes	14.79 del at 2q33	TOP at 26w. Necropsy: l Dandy−Walker variant (DWV), micrognathia, facial dysmorphisms, clenched hands with overlapping thin and tapered fingers, and clinodactyly of the fifth fingers.
Ref. [16] (2014)	Case G3: Oligohydramnios, hypoplastic cerebellum, short femur	NR	NR	46,XX, der(5) t(5;9)(5p13.3;9q33.3)	arr 5p15.33p13.3(0-30,814,401) x 1 *de novo*; arr 9q33.3q34.3(129,771,347 −141,053,475) × 3 *de novo* Walker Warburg syndrome; Tuberous sclerosis 1; Kleefstra syndrome	30.5 del at 5p15 11.3 dupl at 9q33	NR

(*continued on next page*)

Table 5 (*continued*)

Ref. number (yr.)	Case	MA	GA	Karyotype	Microarray result (CNVs)	Size (Mb)	Pregnancy outcome
Ref. [48] (2013)	Case 2: Holoprosencephaly, severe FGR, PE, aortic malposition, gastrointestinal abnormalities, ambiguous genitalia	19yr	31w	46, XY, r(13) [8]/45,XY,-13 [5]	arr 13q31.3-q34 large del	21.6 del at 13q31	TOP at 33w
Ref. [49] (2013)	VSD, hydramnios, FGR, preaxial polydactyly and facial dysmorphisms	29yr	17w	47,XY,+2 [8] /46,XY [22]; QF-PCR on placenta revealed trisomy 2 derived from maternal meiosis I non-disjunction. Interphase FISH on uncultured amniocites, detected 11.1% mosaicism for trisomy 2. Uniparental disomy 2 was excluded by QF-PCR.	arr[hg19] 2p25.3q37.3 (0-242,936,883) × 2.46 mat Trisomy 2	2.46 dupl at 2p25	TOP. Facial dysmorphism and preaxial polydactyly of the hand
Ref. [50] (2013)	Microcephaly, VSD, large overriding vessel with pulmonary artery branching, PTA, DORV	NR	23w	Normal	arr 22q11.21 del	3.08 del at 22q11	TOP at 24w. Necropsy: palpebral fissures, prominent nasal root, bulbous nasal tip, hypoplastic alae nasi, small mouth, micrognathia, small overfolded ears
Ref. [51] (2013)	Holoprosencephaly, premaxillary agenesis	NR	19w	46,XX, del(18)(p11.21)	arr 18p11.32–p11.21 del *de novo* QF-PCR showed mat origin FISH analysis confirmed haploinsufficiency of TGIF gene	14.06 distal del at 18p11	TOP
Ref. [12] (2012)	Facial dysmorphisms, exomphalos, AVSD, DWV and AMC	33yr	26w	47,XY,+18	arr 18p11.32q23	NR dupl	IUFD at 32w.
Ref. [52] (2012)	Micromelia, abnormal spinal curvature, facial dysmorphisms: CP, micrognathia, hypertelorism, marked prenasal thickness, broad and flat nasal bridge, large philtrum with thickened and everted upper lip	NR	30w	Normal	12p13.33p11.1 (RP11-28313?RP11-313F23) × 3 *de novo*	34.21 at 12p13 dupl	IUFD at 32w
Ref. [53] (2012)	CHD, mild lateral ventriculomegaly	NR	20w	46,XY, −14, +der14(q31) *de novo*	46, XY, −14, +der(12; 14) (p13; q32.33) del(14) (q32.33→ qter)	del(14)(q32.33→ qter)	NR
Ref. [54] (2011)	Hypertelorism, epicanthic folds, prominent nose, triangular face, low-set ears, clinodactyly of the fingers and small big toes	37 y	18w	47,XX,+mar [17]/46,XX [15]; Parental karytypes were normal	sSMC was r(4)(p12q13.2) gain in the gene dosage encompassing the region of 4p12/q13.2 a small supernumerary marker chromosome (sSMCs) derived from ring chromosome 4	21.7 gain	TOP
Ref. [55] (2011)	Flat facial profile, hypertelorism, low-set ears, a depressed nasal bridge, clinodactyly, hypoplastic midphalanx of the fifth fingers, brachycephaly and epicanthic folds, polyhydramnios	34 y	20w	46,XX,der(9)t(9;21) (q34.3;q22.11) *de novo* FISH analysis showed that the chromosome 21 segment in the distal end of the long arm of der(9) was of 21q in origin, and the distal subtelomeric region of 9q was not deleted	arr 21q22.11q22.3 (RP11-367F15→RP11-100I21) × 3 mat encompassing the Down syndrome critical region (DSCR) *de novo* arr 21q22.11q22.3 (32,110,552 −46,944,323)	14.8 dupl of distal 21q	TOP. Necropsy: flat facial profile, hypertelorism, low-set ears, depressed nasal bridge, clinodactyly, hypoplastic midphalanx of the fifth fingers, brachycephaly and epicanthic folds

Ref. [56] (2010)	Case 1: Microcephaly with a BPD and a HC < 5th centile, DWM, irregular-shaped skull, nuchal oedema, TGA	42yr	18w	46,XY,der (13) t(7;13)(p15.;q33.3) FISH analyses showed that the chromosome 7 segment in the distal end of the long arm of chromosome 13 was of 7p in origin and the distal end of 13q was deleted	arr 7p22.3p15.3 (RP11-90P13→RP11-34M9) × 3 (Trisomy 7p); arr 13q33.3q34 (RP11-313L9→RP11-450H16) × 1 (partial monosomy 13q (13q33.3→qter), with a triple dose of the TWIST gene and haploinsufficiency of the TWIST gene will cause craniosynostosis	19.9 dupl at 7p22.3→7p15.3; 7.38 del at 13q33→313q34	TOP
Ref. [56] (2010)	DWM, abnormal skull development, microcephaly, nuchal edema and TGA	42yr	18w	46,XY,der(13) t(7;13)(p15.3;q33.3) FISH indicated a translocation between 7p and 13q in the der(13)	arr 7p22.3p15.3 (RP11-90P13→RP11-34M9) × 3; 13q33.3q34 (RP11-313L9-RP11450H16) × 1. *de novo* partial trisomy 18 (7p15.3→pter)	19.9 dupl at distal 7p; 7.38 del at distal 13q	Delivery at 38w; microcephaly, hypertelorism, epicanthal folds, CP, broad flat nose, simian creases, broad hands, tapered fingers, clubfeet, micropenis, sacral dimple, hypotonia.
Ref. [57] (2008)	SCyH, ASD or VSD, abnormal facial features with orbital hypoplasia,bilateral absence of the middle phalanx of the 5th digit, encephalocele, low-set ears, webbed neck	30yr	10w 6 d	[46,XX,der(13) t(2;13)(p25.1;q32)pat] Chromosome 13 del at G-banding and FISH with balance translocation in the father 46,XY,t(2;13)(p25.1;q32)	Copy number gain (RP11-163G21) of 4 BAC clones at 2p25.3 reflecting cryptic 2p25.1→pter trisomy; Copy number loss of 3 BAC clones at 13q34 as a result of the 13q monosomy 13 BAC clone showing loss (RP11-75F3) and the closest clone giving a normal result (RP11-122A8); 2 breakpoint determined by G-banding as 2p25.1 refined the chromosome; 13 breakpoint to a region between BAC clones RP11-122A8 and RP11-151A6 at 13q32.3 2 BAC clones RP11-46A10 at 1q25.3 and RP11-79M1 at 14q23.1: VOUS 13q del syndrome	22 dupl 13 del at 13q34 1.2 del at 13q32.3	TOP at 18w5d.Necropsy: encephalocele, TOF, pulmonic valve stenosis, VSD and overriding aorta.Low-set ears, open and irregular palate and weebed neck.
Ref. [58] (2007)	Case 2: Microcephaly, cerebellar vermis agenesis, CHD	40yr	NR	46,XX,t(7;10)(q11.22;p14)	7q→ter del on derivative chromosome 10 of an inherited maternal reciprocal translocation t(7;10)	NR, del at 7q→ter	TOP
Ref. [59] (2005)	SCyH, epicanthal folds, hypertelorism, broad nasal bridge and low set ears, ambiguous genitalia. At 18 w: fetal hydrops developed.	30yr	16w	46,XY,der(10)t(10;18) (q25.3;q23)pat	Loss of distal 10q, partial monosomy 10q25.3→qter; Gain of distal 18q, partial trisomy 18q23→ter; haploinsufficiency of the critical urogenital developmental region resides on 10q26	NR del at 10q dupl at 18q	TOP at 18w. Necropsy: large SCyH, ambiguos genitalia with a small phallus; hypertelorism, epicanthal folds, broad nasal bridge and low-set ears

Table 6
Microarray results in cases of first trimester increased nuchal translucency (NT).

Ref. number (yr.)	Case	MA	GA	Karyotype	Microarray result (CNVs)	Gain or Loss size (Mb)	Pregnancy outcome
Ref. [31] (2015)	Case 2: NT = 3.8 mm	NR	NR	NR	arr[hg19] 17p12(14,383,794–16,682,433) × 3 (Charcot-Marie-Tooth syndrome, Potocki–Lupski syndrome)	2.3 dupl; 12 OMIM genes	NR
Ref.[31] (2015)	Case 13: ↑ risk of trisomy 21 (1:67)	NR	NR	NR	arr[hg19]1q21.1q21.2(145,417,859 –147,780,609) × 1 *de novo* (1q21.1 del syndrome, #612474)	2.4 del 21 OMIM genes	NR
Ref. [31] (2015)	Case 15: NT = 4.7 mm, ↑ risk of trisomy 21 (1:6)	NR	NR	NR	arr[hg19]17q12(34,816,541–36,328,234) × 1 17q12 del syndrome	1.5 del	NR
Ref. [60] (2012)	↑ NT, micrognathia, distinctive flat profile, small parietal encephalocele, bilateral clinodactyly	24yr	13w	Inconclusive for chromosome 13 monosomy	arr 13[del(13)(q22.2qter)]	NR 40 del	TOP at 14w. Autopsy refused.
Ref. [61] (2007)	↑ NT, skin oedema, micrognathia, AVSD	34yr	14w	46,XX.rev ish dim(12)(p11.;2p13.3); Parents had normal karyotype	arr 12p13.2p11.21(RP11-77I22→RP11-144O23)×1, interstitial *de novo* del	20.1 del at 12p13	TOP at 14w4d.
Ref. [62] (2006)	↑ NT (4.4 mm), SUA. At 22w: nuchal fold (9.5 mm), slightly enlarged fourth ventricle related to partial agenesis or malrotation of the cerebellar vermis, bilateral CPCs, hypertelorism, anteverted nose, abnormal ears.	29yr	12w 22w	46,XX,add(9)(p24.3)	Trisomy 17qter showing a gain of 17q24.3-qter with the chromosome 17q breakpoint mapped between clones RP11-79K13 (17q24) and RP11-300G13 (17q24.3) *de novo* trisomy 17q24.3-pter distal 9p deletion extending from clone CTB-4113 to clone RP11-125B21 *de novo* monosomy 9p24.3-pter arising from a t(9;17)(p24.3;q24.3) translocation	NR Gain of 17q24.3-qter 2.4 9 de at distal 9 pl	TOP at 22w. Necropsy: growth at the 25th centile, high forehead, hypertelorism, short nose with a broad nasal bridge, long philtrum, abnormal ears with a small lobule, broad neck and widely spaced nipples.

Table 7
Microarray results detected in cases of advanced maternal age (AMA)(Legend: HNPP: hereditary neuropathy with liability to pressure palsies).

Ref. number (yr.)	Case	MA	GA	Karyotype	Microarray result (CNVs)	Size (Mb)	Pregnancy outcome
Ref. [31] (2015)	Case 3: AMA	41yr	NR	NR	arr 17q12(34,816,541–36,328,234) × 3 17q12 dupl syndrome *de novo*	1.5 dupl at 17q12	NR
Ref. [31] (2015)	Case 8: AMA	43yr	NR	NR	arr Xp22.31(7,239,742– 7,865,933) × 0 mat; Ichthyosis syndrome	626-kb del	NR
Ref. [31] (2015)	Case 10: AMA	NR	NR	NR	arr 22q11.21(19,172,842– 19,843,647) × 3 pat 22q11.2 dupl syndrome	670-kb dupl	NR
Ref. [31] (2015)	Case 14: AMA	40yr	NR	NR	arr 17p12(14,383,794– 15,475,024) × 1 mat; Hereditary neuropathy with liability to pressure palsies (HNPP)	1.1 del	NR
Ref. [63] (2008)	Case WF24: AMA, family history of trisomy 18	NR	18w1d	46,XX	arr 7q22.1(103,965,388–104,081,539) × 1 pat	NR	NR

polymorphism array (a-SNP) are currently used in genetic prenatal diagnosis. While FISH (fluorescence in-situ hybridization) is an adjunct to conventional karyotyping, especially used to characterize interstitial deletion and/or subtelomeric deletions and duplications [64] a-CGH detects mainly amplifications and deletions and a-SNP detects polymorphism of unique nucleotide (SNP). A SNP may or not cause disease and is not associated with maternal age [65]. CMA can detect variations from 1 kb with a better resolution than the conventional citogenetics karyotype whose resolution is bigger than 5 Mb. The CMA small genomic amplifications and deletions are called CNVs (Copy Number Variants) and these variants increase the chromosomal aberration diagnosis capacity in 15% of cases. CNVs can be classified as pathogenic, likely pathogenic, likely benign, benign and of unknown significance (VOUS) [66]. Some of the millions Variants of unknown significance (VOUS) can be identified and their meaning is not so easy to establish by using chromosomal microarrays. VOUS in specific genetic diseases are associated with an incomplete penetrance or a variable expressivity, therefore in these cases a definitive phenotype cannot be settled, conferring only susceptibility (sometimes not certainly) to a certain disorder which cannot be related to the target investigated disease. Detected VOUS are present in the population and they cannot be trustworthy characterized as benign or pathogenic. VOUS clinical significance can be checked consulting scientific reports. The numbers of VOUS will decrease as their significance is more printed in medical literature. It is suposed that the VOUS rate from 6 years ago can be larger than today, but according to current papers it remains relatively constant, between 1.5% and 0.3%. This interval difference can be expounded as we keep in view the different resolution of microarray platform used by the authors and the variations in the interpretation of the significance of the same VOUS by different counsellors. Opening statistic should be analysed considering the appliance methods and the classification of the VOUS.

Should we include these abnormal findings of a prenatal diagnosis chromosomal microarray (CMA) analysis in the genetic counselling? Some professional groups, working with genetic counselling, describe all the results to the patient's family [7]; on the other hand there are groups that report only findings with known clinical significance [16], particularly the Belgian group, that do so if the CMA changes have a correspondence with the observed ultrasound phenotype [8]. Classification, using familial segregation, has been designed to help geneticists counsellors in the decision how to inform patients the array results [67].

From analysis of the current review about the role of a-CGH in cases of prenatally detected fetal malformations, few conclusion can be drawn about genetic characterization following diagnosis of an abnormal fetal phenotype: 1) obstetricians involved in prenatal diagnosis should accurately report all features of abnormal fetal phenotype, using both 2D and/or 3D/4D ultrasound (where available) as well as fetal-MRI (where available and indicated); a direct communication with the geneticists is highly advocated; 2) it is recommended to record and store digitalized images on an optical disk to be used for subsequent offline analysis or for expert consultation; 3) a-CGH has demonstrated to be a useful technique for investigating small unbalanced chromosomal abnormalities (less than 5 Mb); 4) clinically, it is of paramount importance to select an a-CGH with a high sensitivity (as the capability to detect the vast majority of CNVs) at a low specificity (as the capability to detect VOUS) with resolution of the order of 10 kb in targeted disease-specific regions of the genome; 5) a consensus for a routine inclusion of a-CGH in all cases of fetal karyotyping has not currently been established, therefore eliciting a cost-effective analysis; however it has been reported that the use of microarrays when fetal malformations are seen at ultrasound improve the detection of CNVs or VOUS over conventional cytogenetic analysis [68,69]; 6) congenital heart defects, multiple congenital abnormalities and CNS malformations were the most frequent anomalies associated with a-CGH abnormalities in fetuses with normal or abnormal karyotype at conventional cytogenetic analysis; 7) genetic characterization of congenital anomalies by microarrays has improved the knowledge and the calculation of recurrent risk, enhancing the quality of prenatal counseling by the multispecialist team; 8) it is important to emphasise that a-CGH is not able to detect either single nucleotide mutations or mitochondrial mutations.

Conflict of interest

No conflict of interest

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