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Clinical use of array comparative genomic hybridization (aCGH) for prenatal diagnosis in 300 cases†

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Abstract

Objective—To evaluate the use of array comparative genomic hybridization (aCGH) for prenatal diagnosis, including assessment of variants of uncertain significance, and the ability to detect abnormalities not detected by karyotype, and vice versa.

Methods—Women undergoing amniocentesis or chorionic villus sampling (CVS) for karyotype were offered aCGH analysis using a targeted microarray. Parental samples were obtained concurrently to exclude maternal cell contamination and determine if copy number variants (CNVs) were *de novo*, or inherited prior to issuing a report.

Results—We analyzed 300 samples, most were amniotic fluid (82%) and CVS (17%). The most common indications were advanced maternal age (N = 123) and abnormal ultrasound findings (N = 84). We detected 58 CNVs (19.3%). Of these, 40 (13.3%) were interpreted as likely benign, 15 (5.0%) were of defined pathological significance, while 3 (1.0%) were of uncertain clinical significance. For seven (~2.3% or 1/43), aCGH contributed important new information. For two of these (1% or ~1/150), the abnormality would not have been detected without aCGH analysis.

Conclusion—Although aCGH-detected benign inherited variants in 13.3% of cases, these did not present major counseling difficulties, and the procedure is an improved diagnostic tool for prenatal detection of chromosomal abnormalities.

Keywords

aCGH; chromosomal abnormality; chromosomal microarray analysis; prenatal; copy number variants; CVS; amniotic fluid

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CONFLICT OF INTEREST STATEMENT

The Medical Genetics Laboratories of the Department of Molecular and Human Genetics at Baylor College of Medicine (BCM), where the authors are employed as trainees, staff or faculty members, offers extensive genetic laboratory testing and derives revenue from this activity. BCM currently uses oligonucleotide arrays manufactured by Agilent Technologies.

INTRODUCTION

Prenatal diagnosis of cytogenetic abnormalities has focused primarily on detection of the most common aneuploidy in humans, trisomy 21. The currently accepted approach is to perform a combination of screening procedures in the first and second-trimester based on maternal age, levels of serum analytes, and ultrasound-detected markers or abnormalities in the fetus (Wapner *et al.*, 2003; Malone *et al.*, 2005; ACOG, 2007a; Reddy, 2007). Integrated first- and second-trimester screening detects approximately 95% of fetuses with Down syndrome with a 5% rate for an invasive procedure (Reddy, 2007). Such screening also provides a risk evaluation for trisomy 18, neural tube defects, and more recently Smith–Lemli–Opitz syndrome (Craig *et al.*, 2006; ACOG, 2007a). This strategy is limited by lack of detection of many unbalanced structural chromosomal abnormalities (i.e. deletions and duplications) from submicroscopic chromosomal aberrations below the resolution of a standard karyotype analysis (Stankiewicz and Beaudet, 2007). Such disorders can be diagnosed by locus-specific fluorescence *in situ* hybridization (FISH), but it is not feasible to perform FISH for all possible deletion and duplication syndromes, or to identify which pregnancies are at increased risk with any regularity. In general, neither family history nor advanced maternal age can help guide the clinical indication for testing.

Array-based comparative genomic hybridization (aCGH) allows for fast and accurate detection of unbalanced structural and numerical chromosomal abnormalities (Pinkel *et al.*, 1998; Mohammed *et al.*, 2001; Vissers *et al.*, 2003; Barrett *et al.*, 2004). While experience with diagnostic aCGH in the pediatric population is extensive (Bejjani *et al.*, 2005; Cheung *et al.*, 2005; Shaffer *et al.*, 2006; Lu *et al.*, 2007; Stankiewicz and Beaudet, 2007), experience with its use for clinical prenatal diagnosis is still limited. Rickman and colleagues hybridized DNA extracted from amniotic fluid (AF) and chorionic villus sampling (CVS) cultures with known cytogenetic abnormalities and confirmed the abnormality in 22/30 cases using a whole-genome bacterial artificial chromosome (BAC) array and in 29/30 cases using a clinical ‘targeted’ array (Rickman *et al.*, 2005). The first prospective validation study on 98 AF and CVS samples demonstrated a 5% detection rate of clinically significant abnormalities (Sahoo *et al.*, 2006a). Other smaller studies have investigated the use of aCGH for analysis of cell-free DNA in amniotic fluid (Larrabee *et al.*, 2004; Miura *et al.*, 2006; Lapaire *et al.*, 2007).

Here we present our experience with prenatal diagnosis on DNA extracted from CVS or AF using a targeted clinical CGH array on 300 cases. Our data indicate that cytogenetic abnormalities are readily detected. We confirm the value of aCGH for characterization of small supernumerary marker chromosomes (sSMCs) of unknown origin and for the detection of submicroscopic copy number changes in cases with apparently balanced structural chromosomal abnormalities or with a normal karyotype.

METHODS

Patients and samples

Samples included in this dataset were received between September 2005 and February 2008 for clinical prenatal aCGH testing by the Baylor College of Medicine Medical Genetics Laboratories. Patients underwent pretest counseling as described (Darilek *et al.*, 2008) and provided informed consent for adjunct aCGH testing. We received direct CVS, uncultured AF, or established cultures of such samples, as well as one fibroblast culture from a fetal skin biopsy and one cystic hygroma fluid sample. Blood samples from both parents were requested with the fetal sample to test for possible maternal cell contamination (MCC) and for expedited characterization of potential familial copy number variants (CNVs) where

necessary. Blood samples were received from both parents in 293 (94.8%) of cases, from the mother only in 15 (4.9%) cases, and from neither parent in 1 (0.3%) case. This report does not include data on 98 samples from our validation study (Sahoo *et al.*, 2006a), but includes 8 samples described in a report on a novel DNA extraction method from uncultured AF (Bi *et al.*, 2008).

Cell culture and DNA extraction

G-banded karyotypes and, if requested, FISH, to detect aneuploidy for chromosomes 13, 18, 21, X, and Y were performed on all samples using standard protocols, either in the referring laboratory or in our laboratory. Typically, 25–30 mg of CVS tissue or 25 mL of AF (at a gestational age of at least 16 weeks), were required if both karyotype analysis and aCGH were performed in our laboratory. When karyotype analysis was performed in the referring laboratory, 15 mg of CVS and 15 mL of AF were required. Direct analysis was performed on uncultured CVS (5 mg) or AF (5–7 mL) if a sufficient amount of sample was submitted. Backup cultures were established with the remainder of the samples to perform confirmatory karyotype, FISH or aCGH studies if needed. High molecular weight DNA was extracted from amniotic fluid cell pellets, chorionic villi or cultured cells using the QIAamp DNA Blood Midi Kit (Qiagen, Valencia, CA) with minor modifications. For samples with limiting amounts of DNA, 10–30 ng each of the fetal DNA and reference DNA were subjected to whole-genome amplification (WGA) using the GenomePlex WGA kit (Sigma-Aldrich Corporation, St. Louis, MO). All fetal DNA samples were tested for MCC using the PCR-based Identifiler system (AmpFLSTR, Applied Biosystem, Foster City, CA). Prior to labeling and hybridization, fetal gender was determined by amelogenin gene polymerase chain reaction (PCR) amplification. Test DNA and reference DNA of the same gender were then cohybridized to the array. Reference DNAs were derived from peripheral blood leukocytes of a phenotypically normal male and female.

Array platforms

Targeted clinical BAC arrays were used for 190 samples, including the Baylor College of Medicine (BCM) BAC Chromosomal Microarray V5 or 6; the others were hybridized to V6 of the BCM oligonucleotide Chromosomal Microarray. Arrays were designed to provide redundancy with high sensitivity and specificity for detection of clinically significant unbalanced chromosomal abnormalities, while minimizing detection of benign CNVs or CNVs of uncertain clinical significance. Manufacturing, composition and clinical application of these arrays have been previously described (Cheung *et al.*, 2005; Roa *et al.*, 2005; Sahoo *et al.*, 2006b; Lu *et al.*, 2007). The BCM V5 BAC array contained 853 distinct clones, targeted to cover 75 genomic disorders, 41 relevant subtelomeric regions with an average of 10 clones per region, and 43 unique pericentromeric regions. The BCM V6 BAC arrays contained 1476 distinct clones, covering over 140 genomic disorders, 41 relevant subtelomeric regions, 43 unique pericentromeric regions, and selected backbone clones distributed at an equivalent of a 650 band-interval. The V6 oligonucleotide arrays are custom designed emulations of the V6 BAC array manufactured by Agilent Technologies, Inc. (Santa Clara, CA) (Ou *et al.*, 2008). Upon transition from a BAC to an oligo array platform, the oligonucleotide BAC-emulation design was preferred over more randomly spaced oligonucleotides to assure optimal coverage of clinically relevant regions.

Hybridization and data analysis

DNA labeling and hybridization were performed as described for BAC arrays (Cheung *et al.*, 2005; Sahoo *et al.*, 2006b; Lu *et al.*, 2007). Briefly, two dye-label reversal hybridizations were done for each sample. Microarray image files were quantified using GenePix Pro 5 software, and signal intensities from both hybridizations were normalized and combined to determine a single-fold change value for each clone. Inferences were made for all clones

using these final combined data values. All analyses were performed on $\log_2$ ratios using a code for the normalization and inference implemented in the R statistical programing language (Shaw *et al.*, 2004; Cheung *et al.*, 2005; Sahoo *et al.*, 2006b; Lu *et al.*, 2007).

For the oligonucleotide array, DNA labeling and hybridization were performed according to the manufacturer's protocol (Agilent Technologies). Microarrays were scanned on an Agilent G2565 scanner and image files were quantified using Agilent's Feature extraction software (V9.0). Text file outputs were converted to BAC-level emulation data by combining the oligo aCGH data corresponding to regions encompassed by BAC clones, as described (Ou *et al.*, 2008).

Data interpretation and confirmatory analysis

Detected copy number gains or losses were compared to known CNVs in publicly available databases and in our own database of results from more than 12 000 samples. Parental samples were analyzed by aCGH, specifically to examine the presence or absence of CNVs detected in the fetus. If copy number changes were *de novo* and/or encompassed known genomic disorders, confirmatory FISH studies were also performed on metaphase spreads or interphase nuclei prepared from AF or CVS cultures using standard procedures.

RESULTS

Clinical indications and sample types

A total of 309 samples were submitted (Table 1), 254 (82.2%) AF and 53 (17.2%) CVS. For AF, analysis was performed on cultured amniocytes in 166 cases (65 of which were submitted as established cultures), on DNA directly extracted from AF cell pellets without ($n = 38$) or with ($n = 50$) prior to WGA. For CVS, analysis was performed on cultured villi in 27 (20 of which were submitted as established cultures), on directly extracted DNA in 21, and DNA obtained after WGA in 5 cases. Hence, analyses were completed with uncultured material for 88/189 (46.6%) of AF and for 26/33 (78.8%) of CVS. There was one fetal skin fibroblast culture and one cystic hygroma fluid sample. For two cases, MCC in the submitted sample was detected prior to aCGH analysis. The first was a direct AF for which results were reported from the amniocyte culture after it was found to be free of MCC. The second was an established CVS culture and follow-up amniocentesis was required to complete both the fetal aCGH and karyotype analysis.

The indications that were recorded ($N = 367$; Table 2) included one ($N = 249$) or up to three ($N = 115$) of the following: advanced maternal age of over 35 years at time of delivery ($N = 123$; 33.5%), abnormalities detected on prenatal ultrasound ($N = 84$; 22.9%), a previous child with or another family history of a genetic disorder or chromosomal abnormality ($N = 87$; 23.7%), further work-up for a chromosomal abnormality detected on the fetal karyotype ($N = 28$; 7.6%), parental concern ($N = 33$; 9.0%), abnormal maternal serum screening results ($N = 9$; 2.5%); unspecified ($N = 3$; 0.8%). For 17% of patients, more than one indication was recorded. The ultrasound abnormalities included both major and minor abnormalities, ranging from severe congenital heart defects to such anomalies as an isolated choroid plexus cyst (Table 2).

Likely benign copy number changes

Of the total 309 submitted samples, 9 were not analyzed for reasons unrelated to the aCGH procedure (insufficient quality of the submitted cultured samples, MCC detected in the submitted sample, or test canceled by the submitter, parental samples unavailable). No deletions or duplications were seen in 242 (80.7%) of the remaining 300 samples. Copy number changes were detected in 58 (19.3%). Of these, 40 (13.3%) were CNVs that were

interpreted as likely benign and of no clinical significance (Table 1, see Supporting Information). Of these, 39 were inherited from a phenotypically normal parent and 1 was *de novo*, but had been previously seen multiple times in our database of over 12 000 aCGH hybridization results from all previously analyzed clinical cases and phenotypically normal individuals.

Copy number changes of established or uncertain clinical significance

Eighteen (6.0%) copy number changes (Table 3) were either of established clinical significance, although of varying severity and penetrance ($n = 15$; 5.0%) or of uncertain clinical significance ($n = 3$; 1%). Two of the 18 were *de novo* CNVs, detected in the fetal DNA but not in parental DNAs, and not found in public CNV databases or in the laboratory's aCGH database. The laboratory did not perform independent assessments of paternity in these two cases, but assumed paternity as described. The first *de novo* case (Table 3, case no. 7; Figure 1(A)) was referred because of micrognathia and micropenis detected on prenatal ultrasound in a male fetus with a 46,XY karyotype. A *de novo* copy number loss in the fetal DNA, not present in the mother's DNA, of one clone in Xq27.3 was identified by aCGH, but not by FISH, as can be the case for smaller CNVs. The clinical significance of this CNV is uncertain, but the deleted region does not contain any genes, and it likely was not the cause of the phenotype. This result did not influence the pregnancy management. The infant died 1 day after birth and had a diaphragmatic hernia, ambiguous genitalia, scalp edema, a two-vessel cord, and a suspected clinical diagnosis of Fryns syndrome. The second *de novo* case (Table 3 case no. 11; Figure 1(B)) was analyzed because of fetal polydactyly detected on prenatal ultrasound, and showed a deletion containing known genes in 15q26.3 of at least 800 kb. The result was interpreted to possibly be the cause of the polydactyly, bearing an increased risk for other disabilities not detectable on prenatal ultrasound. It contributed to the parents' decision to terminate the pregnancy; additional fetal samples for follow-up studies were not available. Although it can be argued that this aCGH finding is likely of clinical significance, we more cautiously classified it as 'uncertain'.

The third case with a result of uncertain clinical significance (Table 3 case no. 18) showed a large (~5.1 Mb) deletion in 3p26.2–p26.3, detected both by karyotype and aCGH, that was also present in the mother who has no reported medical problems. The 15 remaining cases were interpreted as 'of established clinical significance', although the phenotypic consequences were not always fully predictable (e.g. for sSMCs).

Copy number abnormalities were further detected in eight samples that were submitted for work-up of a cytogenetic abnormality found on prior fetal karyotype analysis (Table 3 case nos. 1, 3, 4, 5, 9, 12, 13, 18). There were four cases (Table 3 case nos. 2, 15, 16, 17) with whole-chromosome aneuploidy (one 47,XX, +21, two 45,X, and one 47,XYY). Those were equally well detected by aCGH and karyotype analysis, but the aCGH data were available before the karyotype result for the fetus with trisomy 21. We are not aware of any aCGH failure to detect abnormalities known to be present based on karyotype or FISH, except for cases where aCGH analysis was requested to investigate the origin of a *de novo* sSMC found on the fetal karyotype. Out of nine samples that were submitted for this indication, there were four (Table 3 case nos. 1, 4, 5, 13) with a gain of one or several BAC clones on specific chromosomes, resulting in the diagnosis of two ring chromosomes 12, one ring chromosome 21 and a bisatellited marker containing material from the 22q11 cat-eye syndrome critical region. When aCGH did not detect genomic imbalance in the presence of a marker chromosome, we reported that counseling could be more reassuring, whereas for those with copy number gain, we reported that the presence of the *de novo* sSMC could result in an abnormal phenotype.

In one case, aCGH confirmed a duplication in 8p22 initially detected on the fetal karyotype (Table 3 case no. 12); this pregnancy was terminated after counseling. There were four deletions or duplications for which the pregnancy was known to be at elevated risk by virtue of maternal carrier status (*MECP2* duplication, *PMD* duplication, *STS* deletion, or balanced translocation; Table 3 case nos. 6, 8, 10, and 14, respectively). There were two cases where aCGH provided a diagnosis that would have otherwise remained unascertained. In one (Table 3 case no. 3) previously reported case (Simovich *et al.*, 2007), aCGH was performed to determine copy number balance at the breakpoints of a *de novo* apparently balanced (2;9) translocation and revealed a deletion at the 9q34 breakpoint, indicating that the fetus was affected with the 9q34 deletion syndrome (Yatsenko *et al.*, 2005). A second (Table 3 case no. 9) was submitted to investigate a mosaic add(1)(p22), add(11)(p15) chromosomal abnormality, detected in a cultured CVS sample. While this finding was ultimately shown to be a culture artifact, the aCGH identified an unsuspected deletion in 1q21. This deletion was *de novo* and overlapped with the deleted region associated with thrombocytopenia-absent radius (TAR) syndrome (Klopocki *et al.*, 2007; Uhrig *et al.*, 2007). A targeted ultrasound was recommended and revealed bilaterally absent radii in the fetus, consistent with this diagnosis.

DISCUSSION

We found that aCGH yielded new clinically relevant results in 7 (2.3%) of 300 (1/43) analyses, that provided additional information for prenatal genetic counseling and risk assessment. These cases included four marker chromosomes that were rapidly identified, the discovery of a 9q34 deletion in an apparently balanced translocation, a case of TAR syndrome and a deletion of at least 800 kb in 15q26.3. It is virtually certain that the 9q34 deletion (Yatsenko *et al.*, 2005) and the 1q21 (TAR) deletion (Klopocki *et al.*, 2007) are associated with a clinical disorder. Although not proven, and therefore classified as 'uncertain', it is also likely that the *de novo* 15q26.3 deletion is associated with a clinical phenotype.

In two of these seven cases (0.7% or 1/150; 9q34 and 1q21 TAR syndrome deletion), aCGH detected a known disorder that would not have been found if only a karyotype or aneuploidy FISH had been performed. This risk of 1/150 is equivalent to the term pregnancy risk for Down syndrome of a 38-year-old woman, and to the term risk for all common aneuploidies of a 36-year-old woman (ACOG, 2007b). This risk is also higher than the currently quoted risk of a procedure-related loss after amniocentesis of 1/300–1/500, and the similar risk of loss after CVS (ACOG, 2007b).

We identified the origin of four out of nine sSMC. The quoted risk for an abnormal phenotype is 14.7% for nonsatellited sSMCs, and 10.9% for satellited sSMCs (Warburton, 1991). Determining the origin of sSMCs is important for accurate genetic counseling, but can be laborious and difficult by conventional cytogenetic methods and FISH. If the genetic material contained in the marker is represented on the array, i.e. the marker contains euchromatic material, it should show a copy number gain for this region. Although a high percentage of SMCs (59%) are mosaic (Crolla *et al.*, 2005), levels of mosaicism as low as 15%, inclusive of SMCs, can be detected by aCGH (Ballif *et al.*, 2006; Ballif *et al.*, 2007; Cheung *et al.*, 2007). In our study, in the four cases in which the sSMCs were detected by aCGH, the percent of cells in the cultures that contained the sSMC ranged from 33 to 100%. In the five cases in which the sSMCs were not detected, mosaicism ranged from 25 to 85% in the initial culture. Of note, three of these (67, 83, and 85% mosaicism) were karyotypically determined to be bisatellited markers and confirmed by FISH analysis to originate from chromosomes 14/22 and 13/21. Markers derived from these acrocentric chromosomes are occasionally seen in normal individuals and have historically been

predicted to contain only heterochromatin. Since our array was targeted to unique (euchromatin-containing) pericentromeric sequences, SMCs that contain only heterochromatin material would not be expected to be detectable using this methodology. Therefore, a normal result by aCGH provides reassurance that the marker is likely benign.

An abnormal ultrasound finding was the sole indication for the aCGH analysis in only four of the cases that proved to be abnormal (Table 3 case nos. 7, 11, 15, 17). Furthermore, the limb abnormalities in the TAR case were detected after the aCGH result became available. This suggests that reserving prenatal aCGH analysis for pregnancies with an abnormal prenatal ultrasound may not be the optimal diagnostic strategy. This is not surprising because many of the genomic disorders represented on the targeted array are not associated with clinical features that are detectable on prenatal ultrasound. For some, such features may be present, but are atypical or variable (e.g. congenital heart defect or intra-uterine growth restriction). Moreover, it has been shown that genome-wide arrays detect pathological abnormalities in up to 17% of patients with mental retardation (Shaw-Smith *et al.*, 2004; de Vries *et al.*, 2005; Schoumans *et al.*, 2005; Friedman *et al.*, 2006; Krepischi-Santos *et al.*, 2006; Lugtenberg *et al.*, 2006; Menten *et al.*, 2006; Rosenberg *et al.*, 2006) and in 10–27% of children with autism (Jacquemont *et al.*, 2006; Sebat *et al.*, 2007). These conditions are not typically associated with birth defects detectable by prenatal ultrasound.

There is understandable concern that CNVs of uncertain significance might lead to termination of normal pregnancies. Thus, an important question is how often such a finding will be detected by aCGH used for prenatal diagnosis. Our arrays were focused on disease regions and likely to give a lower rate of abnormalities of uncertain significance than would be expected with more complex tiling arrays. We had three such findings (1%) with variable uncertainty. In addition to the 15q26.3 deletion, there was the Xq27.3 *de novo* deletion, which was likely coincidental with the diagnosis of Fryns syndrome, but a causative role cannot be excluded with certainty. The 3p26 deletion, although inherited, was classified as uncertain based on its larger size and possibly variable penetrance of a phenotype. The deleted region contains 11 known genes, and as reviewed by Barber (Barber, 2008), copy number loss of this region has been observed with (Dijkhuizen *et al.*, 2006; Malmgren *et al.*, 2007) and without a clinical phenotype, particularly in a case assessed through prenatal diagnosis (Knight *et al.*, 1995). However, the majority of detected CNVs were smaller, present in a healthy parent and relatively common (13.3%). Most were also prevalent in the normal population, and hence, can be assigned a low risk with relative confidence. This group included ten deletions, four of regions containing known genes, but with no other evidence for benign CNVs, that could be considered of slightly elevated risk. Six were of regions without genes or with genes, but frequently deleted in the normal population. There were 30 duplications, 6 of regions containing known genes, but with no other evidence for benign CNVs, and 24 of regions without genes or with genes, but frequently duplicated in the normal population (Supporting Information Table 1). The experience with over 12 000 pediatric blood samples from patients with disabilities and normal controls is extremely helpful in interpreting common CNVs. However, the possibility that some have reduced penetrance for a disability must be considered. For example, one of the presumed benign variants, a duplication in the region of the Kallmann gene on Xp22.3 found in a male fetus and as a heterozygous change in his mother, was slightly problematic the first time it was detected. It was then interpreted to be of relatively low risk, but with some uncertainty. Subsequently, this duplication was observed in phenotypically normal males, indicating that it was a benign CNV.

Finally, results were obtained from uncultured amniocytes in 46.6% of submitted AF, and from uncultured chorionic villi in 78.8% of submitted CVS.

CONCLUSION

We found that aCGH reliably detected clinically significant copy number changes in 5.0% of fetal samples, confirming previous data (5.1%) from a smaller validation study (Sahoo *et al.*, 2006a), while detection of CNVs of uncertain clinical significance remained at an acceptable low rate (1%). We believe that genetic counseling that informs prospective parents of the additional benefit of aCGH and also of the potential detection of a CNV of uncertain significance should precede all aCGH testing (Darilek *et al.*, 2008). Larger studies will be needed to determine if aCGH will become the first-line test to detect chromosomal abnormalities in fetal samples and to establish whether the improved overall detection rates of clinically significant chromosomal abnormalities will justify offering aCGH more universally to all pregnant women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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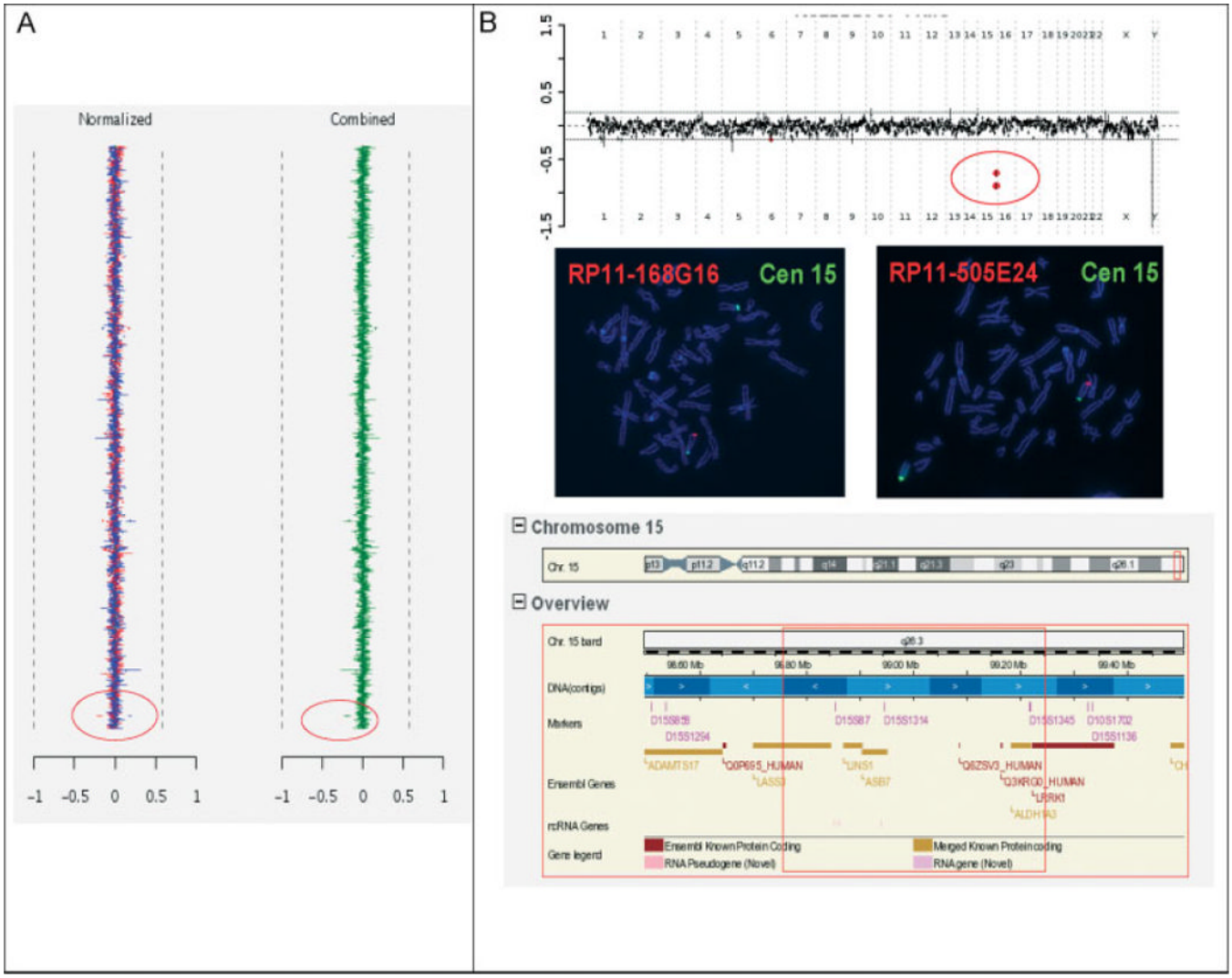

Figure 1.

Copy number losses of uncertain clinical significance. (A) BAC aCGH result on a fetal sample, showing *de novo* copy number loss of one BAC clone in Xq27.3 (Table 3 case no. 7). Normalized data from both dye-reversal hybridizations are shown on the left in red and blue and combined data are shown on the right in green. The red circles indicate the clone affected by copy number loss. (B) Top panel: Oligo aCGH result showing two deleted clones in 15q26 (Table 3 case no. 11) (indicated by the red circle); middle panel: FISH results on metaphase spreads with the deleted clones; bottom panel: schematic of the genomic region from the Ensembl genome browser showing gene content of the deletion (red square)

Table 1

Analyzed samples

Sample type (*N* = 309)		DNA preparation	
Amniotic fluid	254 (82.2%)		
Uncultured amniotic fluid	189/254 (74.4%)	Direct	38/189 (20.1%)
		WGA	50/189 (26.5%)
		Culture	101/189 (53.4%)
Amniocyte culture	65/254 (25.6%)	Culture	65/65 (100%)
Chorionic villus samples	53 (17.2%)		
Uncultured CVS	33/53 (35.2%)	Direct	21/33 (63.6%)
		WGA	5/33 (15.2%)
		Culture	7/33 (21.2%)
CVS culture	20/53 (64.8%)	Culture	20/20 (100%)
Fibroblast culture	1 (0.3%)	Culture	1 (100%)
Cystic hygroma fluid	1 (0.3%)	Culture	1 (100%)

WGA, whole-genome amplification performed prior to DNA labeling.

Table 2

Indications for invasive prenatal diagnostic procedure

Indication	*N* w/o other indications[a]	*N* with other indications[b]	Total *N*[c] (%[#])	% of all 367 indications[d]
Advanced maternal age (≥ 35 years at delivery)	87	36	123	33.5
Abnormal US	67	17	84	22.9
> 2 abnormalities detected			32 (38.1)	
1 anomaly detected			21 (25.0)	
IUGR only			3 (3.6)	
Increased NT only			4 (4.7)	
CPC only			1 (1.2)	
Single umbilical artery only			3 (3.6)	
Unspecified			20 (23.8)	
Family history of genetic or other abnormality	46	41	87	23.7
Chromosomal abnormality detected on prenatal karyotype in current pregnancy	22	6	28	7.6
Parental concern	24	9	33	9.0
Abnormal maternal serum screening result	3	6	9	2.5
Unspecified	3	—	3	0.8
Total of 367 indications●	249	115	367	
Total of 300 analyses	249 (83%)	51 (17%)	300	

Some patients had more than one indication, hence 4 columns are shown:

[a] listed indication was the only reason for the invasive procedure;

[b] listed indication was one of two or more indications;

[c] total for each indication;

[d] % of total for each indication;

[#] % refers to total cases with abnormal ultrasound.

Table 3

Abnormalities of defined or uncertain clinical significance detected by aCGH

Case No.	Array	Indication	aCGH result	Other result	Interpretation/Outcome
1	V5	Abnormal fetal karyotype, unknown sSMC: 47,XX, +mar[12]/46,XX[3]	arr cgh 12q12(RP11-79013,RP11-242B24)x3	ish r(12)(p11q11)(D12Z3+)[16]/12(p11q11)(D12Z3x2)[4]	Female fetus with mosaic ring chromosome 12. Pregnancy continued.
2	V5	Advanced maternal age	arr cgh 21(14 BAC)x3	47,XX, +21	Female fetus with Down syndrome due to trisomy 21. Pregnancy terminated.
3	V5	Apparently balanced *de novo* translocation on fetal karyotype: 46,XY,t(2;9)(q11.2;q34)	arr cgh 9q34.3(RP11-432J22 > RP11-974F22)x1dn	ish der(9)t(2;9)(q11.2;q34) (D9S325-)	Male fetus with 9q34 deletion syndrome. Pregnancy terminated.
4	V6	Abnormal fetal karyotype, unknown sSMC: 47,XX, +mar[11]/46,XX[9]	arr cgh 12p11.22(RP11-847A19 > RP11-8P13)x3	nuc ish 12cen(D12Z3x3), 12p11.2(RP11-8P13x3)[5]/ 12cen(D12Z3x2), 12p11.2(RP11-8P13x2)[195]	Female fetus with mosaic ring chromosome 12. Pregnancy continued. Development appropriate at 6 months of age. Karyotype on blood after birth confirmed r(12) in 7/10 cells.
5	V5	Abnormal fetal karyotype, unknown sSMC: 47,XY, +mar	arr cgh 21q21.1(RP11-625C23, RP11-840D8, RP11-143A3)x3	nuc ish 21q11.2q21.1(RP11-625C23x3,RP11-840D8x3)	Male fetus with ring chromosome 21 confirmed at birth. Pregnancy continued.
6	V5	Maternal carrier of *MECP2* region duplication (Xq28)	arr cgh Xq28(RP11-157E12- > RP4-671D9)x3mat	arr cgh Xq28(RP11-157E12- > RP4-671D9)x3 in mother	Female fetus with maternally inherited heterozygous duplication in *MECP2* region. Pregnancy continued
7	V5	Micrognathia, micropenis, 46,XY	arr cgh Xq27.3(RP11-387H19)x0dn (Figure 1(A))	ish Xq27.3(RP11-387H19)x1; maternal aCGH normal; region does not contain any known genes	Male fetus with *de novo* deletion Xq27.3. Pregnancy continued. Neonatal demise with diaphragmatic hernia, 2-vessel cord, scalp edema, suspected Fryns syndrome (Xq27 deletion likely not the cause of the phenotype).
8	V6 Oligo	Maternal carrier of *PMD* gene duplication	arr cgh Xq22.2(RP11-1123D8- > RP11-832L2)x2mat	nuc ish Xq22(*PLPx2*)	Male fetus affected with Pelizaeus Merzbacher disease. Pregnancy terminated.
9	V6 Oligo	Maternal balanced translocation (10;17) and mosaic unbalanced	arr cgh 1q21.1(RP11-315I20- > RP11-102F23)x1dn, 11p15.5(RP5-998N23- > RP11-38L8)x1 on CVS	ish del(1)(q21.1q21.1)(RP11-769J20-),del(11)(p15.5p15.5) (RP11-38L8)dn on CVS; ish del(1)(q21.1q21.1)(RP11-769J20-)on AF	Male fetus with TAR syndrome possibility on aCGH results, followed

Case No.	Array	Indication	aCGH result	Other result	Interpretation/Outcome
		chromosomal abnormality found in CVS: 46,XY,add(1)(p22), add(11)(p15)[8]/46,XY[2]			by ultrasound confirmation of TAR (del 11p 15.5 on CVS was culture artifact). Pregnancy continued, diagnosis confirmed after birth.
10	V6 Oligo	Maternal carrier of STS gene deletion (Xp22.31)	arr cgh Xp22.31(RP11-483M24- > RP11-143E20)x0mat	46,XY.ish Xp22.3(STS-)	Male fetus with steroid sulfatase deficiency. Pregnancy continued, diagnosis confirmed after birth.
11	V6 Oligo	Abnormal ultrasound: Polydactyly	arr cgh 15q26.3(RP11-168G16- > RP11-505E24)x1dn (Figure 1(B))	46,XY.ish del(15)(q26.3q26.3) (RP11-168G16-,RP11-505E24-)dn	Male fetus with 15q26.3 *de novo* deletion of at least 800 Kb, confirmed by FISH. Pregnancy termination, no sample available for confirmatory testing.
12	V6 Oligo	Advanced maternal age; abnormal fetal karyotype: 46,XY,?dup(8)(p21.3p23.1)	arr cgh 8p22(RP11-520F7, RP11-90I3)x3	nuc ish 8p22(RP11-90I3x3)	Male fetus with duplication 8p22. Pregnancy termination for chromosomal abnormality.
13	V6 Oligo	Abnormal fetal karyotype, unknown sSMC: 47,XX, +mar/46,XX	arr cgh 22q11.1q11.2(RP11-48E19- > RP11-466H9)x3	nuc ish 22q11.21(RP11-9106x3)[66]/22q11.21(RP11-9106x2)[134]	Female fetus with marker chromosome containing three clones from 22q11, in cat-eye syndrome critical region. Pregnancy terminated.
14	V6 Oligo	Cryptic translocation in mother 46,XX,t(8;12)(p23.1;p13.31).ish t(8;12)(D8S504-,VIJTYAC 14+; VIJTYAC 14-,D8S504+)	arr cgh del(8)(p23.1p23.2) (RP11-16G12- > RP11-91J19)x1,dup(12) (p13.31p13.33), (RP11-69M1- > 598F7)x3	Retrospective evaluation showed 46,XY,der(8)t(8;12)(p23.1;p13.31)mat on fetal karyotype, confirmed by arr cgh	Male fetus with 8;12 unbalanced translocation. Fetal demise. Cotwin had same cryptic translocation as mother. Pregnancy continued.
15	V6 Oligo	Abnormal ultrasound: Twin pregnancy, fetal hydrops with cystic hygroma in this twin	arr cgh X(123 BAC)x1, 8p23.1(RP11-241I4)x1	45,X but found XYY by aneuploidy FISH in cystic hygroma fluid (16/19 cells). Mother normal in 8p23.1, father was not available	Female fetus with 45,X. Subsequent IUFD. Pregnancy continued (see case 16).
16	V6 Oligo	Abnormal ultrasound(see #15): Twin pregnancy, normal appearing ultrasound for this twin	arr cgh Y(12 BAC)x2	47,XYY on amniotic fluid karyotype. Follow-up FISH on uncultured whole blood after birth: nuc ish Xcen(DXZ1x1), Ycen(DYZ3x2)[141]/Xcen (DXZ1x1)[30]/Xcen(DXZ1x1), Ycen(DYZ3x1)[29] and 47,XYY[20] karyotype	Male fetus with 47,XYY/45,X/46,XY mosaic karyotype. Pregnancy continued, preterm delivery after premature rupture of membranes.
17	V6 Oligo	Abnormal ultrasound: hypoplastic left heart	arr cgh X(123 BAC)x1 (arr cgh was performed against male reference DNA).	Y chromosome present by PCR to determine fetal gender prior to array; 45,X on fetal karyotype; FISH 45,X[94]/46,XY[6]	Fetus with 45,X/46,XY mosaicism.

Case No.	Array	Indication	aCGH result	Other result	Interpretation/Outcome
					Pregnancy continued. Neonate with mild Turner syndrome phenotype (webbed neck, low hairline, wide-spaced nipples) and hypoplastic left heart.
18	V6 Oligo	Abnormal fetal karyotype: 46,XX,del(3)(p26).ish del(3)(pter-)mat	arr cgh 3p26.3p26.2(RP11-385A18- > RP11-129J10)x1mat, Xq12(RP11-349K4)x3pat	~5.1 Mb deletion in 3p26.2-3p26.3; also present in the apparently normal mother. FISH previously performed by referring lab.	Female fetus with maternally Inherited ~5.1 Mb deletion. Mother appears phenotypically normal. Fetus also with likely benign paternally inherited duplication in Xq12. Normal targeted prenatal ultrasound at 22 weeks gestation. Pregnancy continued.