Chromosomal microarray analysis on uncultured CVS can be complicated by confined placental mosaicism for aneuploidy and microdeletions

Running title: Confined placental mosaicism in prenatal array CGH

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WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

Chromosome preparations from intact villi are known to harbor a high frequency of discrepant results. However, CMA performed prenatally on CVS biopsy often relies on the analysis of DNA extracted from intact villi in order to facilitate rapid turnaround time.

WHAT DOES THIS STUDY ADD?

In addition to the well-known mosaic aneuploidy, mosaic submicroscopic CNVs detected by CMA on uncultured CVS have also been observed and should be carefully interpreted.

ABSTRACT

Objective This study aims to establish the incidence and implications of CPM in the context of prenatal CMA.

Methods We retrospectively reviewed prenatal array data on 1382 consecutive CVS specimens spanning the past 6 years, focusing on those for which whole CVS biopsy (both cytotrophoblast and mesenchymal cells) was used for CMA and cultured cells (primarily mesenchyme) was also analyzed or AF/newborn blood was used for confirmation, to determine the frequency of mosaic abnormal findings that were the result of CPM.

Results Out of a total of 1382 consecutive CVS cases, we identified 42 ($42/1382 = 3.0\%$) cases with abnormal array findings suggestive of mosaicism. Among them, 10 cases were unequivocally interpreted as CPM based on a normal AF/newborn blood confirmatory result. In addition, another 10 cases were interpreted as provisional CPM based on normal results on cultured cells. Notably, 40% (8/20) of the cases revealed complex findings, including multiple mosaic aneuploidies, mosaic submicroscopic copy number variation (CNV) and mosaic aneuploidy plus mosaic CNV.

Conclusion Abnormal CMA results from CVS specimens should be interpreted with caution when mosaicism is evident or suspected. Furthermore, confirmatory testing on amniotic fluid, which contains cells derived from the fetus, is recommended in these cases.

INTRODUCTION

Confined placental mosaicism (CPM) in chorionic villus sampling (CVS) tissue is a well-known phenomenon in prenatal cytogenetic diagnosis, detected in approximately 1-2% of viable pregnancies at 9-12 weeks of gestation (1-6). Depending on the differentiation stage at which the mosaicism occurred, the abnormal cells can reside only in extra-fetal tissues (e.g. the placenta), only in the fetus, or in both. Therefore, interpretation of results demonstrating mosaicism remains one of the most challenging tasks in prenatal diagnosis using CVS tissue. However, due to the advantages provided by CVS through earlier diagnosis of abnormalities (CVS is typically done at 10-13 weeks' gestation, and amniocentesis is done at 15-20 weeks' gestation), it remains a preferred test method for many prospective parents.

CPM involving only the placental cell lineage, which is the source material for CVS procedures, can be divided into three categories: abnormal cells confined to the cytotrophoblast layer (type I, direct preparation), confined to the mesenchymal core (type II, long-term culture) or found in both lineages (type III) (7). In large cohort studies, it has been demonstrated that the majority of the chromosomal mosaicism detected in CVS is CPM without fetal involvement (e.g. 71.85% in (8), 87.24% in (9), 86.5% in (10)).

Chromosomal microarray analysis (CMA) has become a routine prenatal test in many laboratories (11, 12). Compared to karyotyping, CMA in the prenatal setting has the advantages of rapid turnaround time and increased detection for clinically relevant chromosomal abnormalities. CMA significantly increases the diagnostic yield compared to conventional karyotyping, yet without significantly increasing the frequency of detecting variants of unknown significance (11-13). In addition, it has been demonstrated to be a powerful tool to detect mosaicism at levels as low as 9% (14-16). However, while cytogenetic analysis of the cytotrophoblast layer of CVS tissue is no longer conducted in many laboratories, primarily because of the labor-intensive procedure of requiring both short- and long-term cultures, microarrays are frequently performed on intact CVS tissue that includes this outer cell layer.

In this study, we retrospectively reviewed our database of 1382 uncultured CVS cases to determine the incidence and implications of CPM detected by prenatal CMA. While more than half of the confirmed or provisional CPM cases involved partial or whole chromosome aneuploidies, notably, 40% (8/20) of the cases revealed complex findings, including multiple

mosaic aneuploidies, mosaic submicroscopic copy number variation (CNV) and mosaic aneuploidy plus mosaic CNV.

METHODS

Patients

CVS samples from 1382 pregnancies submitted to our laboratory for CMA testing with or without concurrent chromosome analysis and fluorescence in situ hybridization (FISH) were included in this study. The testing was performed for a variety of indications including advanced maternal age (AMA) (61.6%), abnormal ultrasound findings (27.9%), abnormal maternal serum screening (14.0%), abnormal non-invasive prenatal screening (NIPS) (3.8%), parental concern (6.8%), previous abnormal pregnancy (6.0%), family history (8.8%, e.g. carrier parent or family member with chromosomal abnormality or a single gene / Mendelian disorder), and no indication provided (0.6%). Notably, 31.6% of testing included two or multiple indications mentioned above, therefore the sum of aforementioned percentages are higher than 100%. This retrospective data review was approved by the Baylor College of Medicine Institutional Review Board for Human Subjects Research (Protocol Number H-29583).

Preparation of CVS samples

Uncultured villi containing cytotrophoblast cells and the mesenchymal core were used for CMA when possible, with cultured cells maintained as needed for backup. Villi were manually cleaned of maternal decidua prior to DNA extraction. In the majority of cases, GTG-banded chromosome analysis was performed concurrently on cultured cells using standard methods. Aneuploidy FISH analysis was also performed on the majority of cases using the AneuVysion kit (Abbott Molecular) according to the manufacturer's recommendations. Briefly, cleaned villi were incubated in trypsin EDTA at 37°C for 5 minutes, followed by treatment with 0.075 M potassium chloride and subsequently with fixative (3:1 methanol/glacial acetic acid). Therefore, cells analyzed by Aneuploidy FISH were primarily from the outermost cytotrophoblast layer given the short treatment time in trypsin.

Array CGH analysis

Custom designed Agilent BCM OLIGO V7 or V8 oligonucleotide arrays were performed as previously described (15). Arrays were designed to specifically interrogate clinically significant regions while minimizing detection of benign CNVs or CNVs of uncertain clinical significance. V7 array covered 420 genes plus additional 61 genomic regions with known disease association, while V8 array was exon-focused and covered more than 1700 genes; both V7 and V8 array had an average resolution of 30 kb between probes. Mosaic aneuploidies or CNVs were determined by an in-house analysis program. Briefly, an estimate of the percentage of mosaicism was determined by modeling the fold changes (log test/reference ratio) as a mixture arising from cells with a non-mosaic change and the normal value. The resulting mixing proportion is reported as the percent mosaicism (17). Generally, mosaicism as low as 10-20% has been demonstrated to be consistently detected (18). Some mosaic changes not called by the analysis program were noticed through visual inspection (Figure 2 and Table 1 and 2, Cases 8 and 13). Regarding detection of submicroscopic mosaic CNVs, our previous data using this array platform demonstrated accurate detection of mosaic CNVs ranging in size from 35 kb to 185 kb (percent mosaicism ranged from 16% to 56% by FISH analysis) (19). Thus, we expect the detection rate of mosaic submicroscopic CNVs in this size range to be comparable to that of mosaic aneuploidy. Array findings on CVS samples that were suggestive of mosaicism (an average $\log_2$ ratio markedly below the expected 0.58 for gains or -1.0 for losses) were compared to data obtained from concurrent FISH and/or chromosome studies. Regardless of whether or not discrepancies were observed, follow-up studies on amniotic fluid were recommended.

Maternal cell contamination testing

15 unlinked polymorphic loci were PCR amplified from maternal and fetal specimens. The amplified alleles were separated on an ABI 3730xl or 3100 automated sequencer. Maternal cell contamination is excluded when one or two maternal allele is not present in the fetal specimen. MCC is present when both maternal alleles are present in the fetal sample. Mixed artificial controls of 95%:5% and 90%:10% are used for comparison. For all prenatal CMA studies performed in our laboratory, each specimen and the corresponding maternal DNA were subjected to MCC testing. Specifically, MCC study was performed separately for both intact and cultured villi samples when CMA was conducted on both sample types for a single pregnancy. For all mosaic cases involved in this study, MCC was not detected.

RESULTS

Overall data

We retrospectively reviewed prenatal CMA data on CVS specimens analyzed between January 2010 and January 2017 to determine the frequency of results confirmed to be CPM. Out of 1382 consecutive uncultured CVS cases, we identified 42 with abnormal findings on CMA that were suggestive of mosaicism. Regardless of any additional cytogenetic testing performed on cultured CVS, confirmatory testing on amniotic fluid (AF) was recommended in all of these cases. It was performed for 12 cases. In three of these 12 (25%), the original CVS results were confirmed, and they were therefore interpreted as true fetal mosaicism (TFM, Case 21 to 23, Table 1). In the remaining cases, 4 were interpreted as CPM type II or III based on normal results on AF samples and abnormal mosaic findings from long-term cultured CVS cells (Table 1, Cases 1-4). Case 5 was also interpreted as CPM type II or III based on normal newborn chromosome analysis (Table 1). Five other cases were interpreted as CPM type I based on normal results on both AF and long-term cultured samples (Table 1, cases 6-10). The majority (96%) of the abnormal cytotrophoblast / normal mesenchyme mosaic CVS findings were found to be normal in AF confirmatory studies, although TFM cannot be completely ruled out (20). Therefore, for the remaining 28 cases with abnormal findings on CMA that were suggestive of mosaicism without receiving AF samples, we interpreted an additional 9 cases as provisional CPM type I, based on normal karyotype or CMA results from long-term cultured CVS cells. (Table 2, Cases 11 to 19). One specific case (Table 2, Case 20) demonstrated mosaic trisomy for both chromosomes 10 and 13 based on CMA analysis of intact CVS; however, chromosome analysis on cultured CVS cells showed only the mosaic gain of chromosome 10. Therefore, this case was interpreted as partial provisional CPM (provisional CPMI for mosaic trisomy 13) and was also included in this study.

CPM for multiple aneuploidies

Three cases demonstrated mosaic aneuploidy for more than one chromosome, including a mosaic gain of chromosomes 11, 15 and 20 in Case 9 (Figure 1A), mosaic gain of both chromosomes X and Y in Case 13 (Figure 2A), and a mosaic gain of chromosomes 10 and 13 in Case 20. The mosaic aneuploidies detected in uncultured intact CVS tissue in Case 9 were confirmed to be CPM I following a normal CMA study on AF (Table 1), while the mosaic aneuploidies in Case 13 were interpreted as provisional CPM I due to a normal karyotype in

the long-term cultured CVS cells (Table 2). In case 20, mosaic trisomy 13 was detected only by CMA on intact villi and not in long-term cultured CVS cells. Therefore, this finding was interpreted as provisional CPM I, while the status of the mosaic trisomy 10 remained undetermined due to its presence by CMA and in long-term cultured cells.

CPM of submicroscopic single CNVs

Two cases showed mosaic submicroscopic CNVs by CMA, including a mosaic 0.182 Mb loss at Xq27.3 encompassing the *FMR1* gene in Case 4 (Figure 1B), and a mosaic 1.333 Mb gain at 10p12.33p12.31 encompassing the *PLXDC2* gene in Case 16 (Table 2). In Case 4, the mosaic deletion was not detected in the confirmatory AF sample (CPM II or III), while in Case 16, the mosaic copy number gain was not detected by FISH in long-term cultured CVS cells (provisional CPM I).

CPM of aneuploidy plus a CNV

Two cases revealed mosaic aneuploidy plus a submicroscopic CNV by CMA analysis, including a mosaic gain for chromosome 9 plus a mosaic 0.098 Mb loss at Xp22.31 involving part of the *STS* gene in Case 6 (Figure 1C), and a mosaic gain for chromosome 19 plus a 14.595 Mb loss of 15q25.3q26.3 in Case 15 (Table 2). In Case 6, the mosaic changes were not detected in confirmatory AF sample or long-term cultured CVS cells (CPM I), while in Case 15 the mosaic changes were not detected in long-term cultured CVS cells (provisional CPM I).

CPM of multiple CNVs

In Case 19, CMA analysis of intact CVS tissue revealed three mosaic CNVs on chromosome 8p, including a mosaic 1.629 Mb gain at 8p23.2, a mosaic 0.781 Mb gain at 8p23.2p23.1, and another mosaic 0.457 Mb gain at 8p23.1. CMA analysis on cultured CVS cells showed no evidence of the above copy number changes involving chromosome 8 (provisional CPM I, Figure 1D).

DISCUSSION

Confined placental mosaicism detected at prenatal diagnosis using CVS is a well-documented phenomenon. Chromosome analysis by traditional karyotyping is reported to have mosaicism

detected in 1-2% of first trimester pregnancies (8), and the detection rate using microarray is quite comparable (14). Similarly, in this study we observed mosaicism in 3.0% (42 out of 1382) of the CVS cases using CMA. Confirmatory testing on an AF sample or newborn blood was performed for 13 cases. Of these, 3 revealed the same abnormal findings as found on the CVS sample and were therefore interpreted as TFM. In total, 20 of the 42 (48%) cases of mosaicism detected by microarray analysis of intact CVS were determined to be CPM or provisional CPM based on a normal confirmatory AF result and/or normal findings on long-term cultured CVS cells (Table 1 and Table 2). This rate is lower to previously reported rates based on karyotype analysis (8-10). This may be due to the relatively smaller sample size of our current study, and the lower number of AF samples received for confirmatory testing.

In most of the confirmed CPM cases, mosaicism was detected by our in-house CNV analysis algorithm (21) (mosaic fraction ranged from 0.08 to 0.71, Table 1, refer to METHODS section for the algorithm), with the exception of two low-level mosaic aneuploidy cases, which were recognized by visual inspection of the whole genome plot (Case 8 and Case 13, Table 1, Table 2, Figure 2).

Although rare, CPM of multiple trisomies or multiple aneuploidies has been documented (4, 22). In the current study, three cases demonstrated mosaic aneuploidy for more than one chromosome (Cases 9, 13 and 20, Table 1, Table 2). Notably, in Case 20, although CMA on the intact villi sample detected both mosaic trisomy 10 and 13, only the mosaic gain of chromosome 10 was detected on long-term cultured CVS cells. Therefore, it should be noted that for mosaic multiple aneuploidy cases, different cell lineages from the intact villi might contain different chromosomal abnormalities.

Despite the increased resolution of prenatal CMA studies in recent years, most published cases of mosaic chromosomal anomalies that ultimately turned out to be CPM were whole chromosome or chromosome arm aneuploidies, including autosomal trisomies and sex chromosome aneuploidies (8, 11, 23). While some of the mosaic changes were structural rearrangements, they were large (e.g. >10Mb) and microscopically visible (4, 8, 24). CPM for a submicroscopic CNV, however, has only rarely been reported in the literature. One report describes a case of a mosaic microdeletion (979 kb at bands 2p23.1p22.3, CPM type I), which was identified among 464 cytogenetically normal prenatal samples (13). In another case, an exonic deletion of the *STS* gene on Xp22.31 was reported to be CPM type I. (25). In a third case, a mosaic 22q11.2 deletion was detected through NIPS, which was subsequently

found to be present in two out of three placental biopsy sites, while absent from maternal and postnatal neonatal peripheral blood samples (26).

In contrast to these rare reports, the current study demonstrates five cases with CPM for submicroscopic CNVs (Cases 4, 6, 15, 16 and 19, Table 3). Notably, there were two cases with CPM for both a whole chromosome aneuploidy and a submicroscopic CNV (Cases 6 and 15). In addition, one case with CPM showed three mosaic gains on chromosome 8p, ranging from ~0.5 to ~1.5 Mb in size (Case 19). Since none of the CNV breakpoints reside within low-copy repeats (LCR) or segmental duplications (Table 3), they were unlikely to be generated through non-allelic homologous recombination (NAHR)(27). Future studies may be considered to sequence the breakpoint junctions to nucleotide resolution to reveal the underlying mechanism(s) for the generation of these CNVs.

While larger studies are still needed, these data suggest that CPM for submicroscopic CNVs is a more common phenomenon than previously recognized. It is notable that the detection of CPM for submicroscopic CNVs from intact CVS may have important implications with regard to the recent expansion of commercial NIPS to include microdeletion syndrome regions. Based on our data and that of previous reports (13;22;23), it is conceivable that some positive NIPS results for microdeletions could represent CPM, and this could be a factor to consider when deciding whether confirmation studies should be performed on AF or CVS.

CONCLUSION

The current study underscores that mosaic copy number changes detected by CMA using intact CVS samples require careful interpretation, since the majority do not represent true fetal mosaicism. In contrast to karyotyping, CMA is sensitive to small submicroscopic CNVs and low-level mosaic changes, and in the current study, as high as 40% of the cases (8 out of 20) demonstrated complex CPM findings including multiple aneuploidies and multiple CNVs. Overall, 0.36% of CVS samples analyzed demonstrated submicroscopic or nearly submicroscopic CNVs findings (5 out of 1382, including provisional CPM I cases). To our knowledge, no non-mosaic abnormal CMA results from intact or cultured CVS specimens in our data set were found to represent CPM. These data suggest that, in addition to mosaic aneuploidy, mosaic submicroscopic CNVs detected by CMA on direct intact CVS should

also be interpreted carefully, and confirmation on a different sample type, preferentially on amniocytes, may be warranted (9, 25).

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Table 1. Detailed information on the confirmed CPM or TFM cases identified.

Case No.	Indication	13/18/21/X/Y FISH	Chromosome (culture) Result	CMA Result (intact CVS biopsy unless stated)	Amnio follow-up Result	CMA mosaic fraction	CMA mean Log ₂ ratio	Mosaicism type
1	AMA, previous child with <i>de novo</i> 1.6Mb deletion at 9q34.3	N/A	47,XY,+i(9)(p10)[2]/46,XY[70]	Mosaic +9p	Normal CMA	N/A [†]	0.66 [†]	CPM II or III
2	Abnormal U/S – increased NT	+13	No growth	mosaic +13 (plus 22q11.21 loss)	CMA: 22q11.21 loss G-band: 46,XX	0.19	0.13	CPM II or III
3	AMA, abnormal U/S – increased NT	Normal [§]	47,XX,+i(18)(q10)[9]/46,XX[11]	Mosaic +18q	Normal CMA	0.2	0.14	CPM II or III
4	Abnormal U/S – cystic hygroma	Normal	46,XX	Mosaic 0.182 Mb loss at Xq27.3 (<i>FMRI</i> gene)	Normal CMA	0.6	-0.51	CPM II or III
5	AMA, abnormal maternal serum screen – T21	Normal	47,XY,+2[4]/46,XY[16]	Mosaic +2	N/A [†]	0.07	0.05	CPM II or III
6	Abnormal U/S – increased NT	Normal	46,XY	Mosaic + 9, mosaic 0.098 Mb loss of Xp22.31 (<i>STS</i> gene)	Normal CMA	0.51	0.33	CPM I
7	AMA, abnormal maternal serum screen – T21, abnormal NIPS – T21, previous pregnancy with T13	88% + 21	46,XX	Mosaic +21	46,XX	0.48	0.31	CPM I
8	Abnormal U/S – cystic hygroma	Normal [§]	46,XX	Mosaic +13	Normal CMA G-band: 46,XX	N/A [‡]	N/A [‡]	CPM I
9	AMA	Normal	46,XX	Mosaic +11/+15/+20	Normal CMA	0.57/0.58/ 0.58	0.36/0.37/ 0.37	CPM I
10	AMA, abnormal U/S – increased NT	N/A	46,XY	Mosaic +5	46,XY	0.11	0.08	CPM I
21	Abnormal maternal serum screen – T21, abnormal U/S – increased NT	42% monosomy X	46,XX	Mosaic monosomy X	G-band: 45,X[7]/46,XX[26] Normal FISH	0.49	-0.41	TFM IV
22	AMA	N/A	47,XXY[13]/46,XY[8]	Mosaic +X	FISH: 8% XXY G-band: 47,XXY[4]/46,XY[26]	0.25	0.17	TFM V or VI
23	AMA	Normal [§]	45,X	Mosaic monosomy X (plus <i>de novo</i> 6.7 Mb gain at 14q31.1q31.3)	FISH: 12% -Y G-band: 45,X[2]/46,XY[18] CMA: 6.7 Mb gain at 14q31.1q31.3	0.51	-0.43	TFM V for monosomy X

CMA, chromosomal microarray analysis; CPM, confined placental mosaicism; TFM, true fetal mosaicism; AMA, advance maternal age; N/A, not available; U/S, ultrasound; NT, nuchal translucency; T21, trisomy 21; NIPS, non-invasive prenatal screening; T13, trisomy 13.

[†] For non-mosaic 47,XY,+i(9)(p10) cells, the expected Log₂ ratio would be 1.32 (four copies of chromosome 9p in total).

[§] The discordance between 13/18/21/X/Y FISH and CMA results may be explained by the different cell populations analyzed by these two different methods. Specifically, cells analyzed in Aneuploidy FISH were from the outermost cytotrophoblast layer given the short 5 minutes treatment time in trypsin according to the protocol in our laboratory. In contrast, CMA was conducted on DNA from intact CVS biopsy, which includes all cytotrophoblast cells and the mesenchymal core. Therefore, the mosaic changes detected by CMA in chromosome 18 (Case 3), in chromosome 13 (Case 8) and in chromosome X (Case 23) may not be present (or presented in a level that below the 10% reporting cutoff ratio) in the outermost cytotrophoblast layer in these cases, leading to a normal FISH result.

[¶] Normal chromosome analysis (46,XY) after this child was born.

[‡] Mosaic change not called by the in-house analysis program but noticed by visual inspection. See Figure 2 for CMA plots.

Table 2. Detailed information on the provisional CPM I cases based on normal results of long-term culture (LTC).

Case No.	Indication	13/18/21/X/Y FISH	Chromosome (culture) Result	CMA Result (intact CVS biopsy unless stated)	Amnio follow-up Result	CMA mosaic fraction	CMA mean Log ₂ ratio
11	AMA	Normal [†]	46,XY	Mosaic +13	N/A	0.2	0.14
12	AMA, abnormal U/S – increased NT	42% -X	46,XX	Mosaic -X	N/A	0.47	-0.39
13	AMA	Normal [†]	46,XY	Mosaic +X,+Y	N/A	N/A [‡]	N/A [‡]
14	Parental concern, previous pregnancy with <i>de novo</i> 2.768 Mb gain at 5p15.32p15.2	16% -X	46,XX	Mosaic -X	N/A	0.18	-0.14
15	Abnormal maternal serum screen – T21	Normal	46,XX	mosaic +19, 14.595 Mb loss of 15q25.3q26.3	N/A	0.08/0.72	0.06/-0.64
16	AMA	Normal	46,XY [§]	Mosaic 1.333 Mb gain of 10p12.33p12.31	N/A	0.43	0.28
17	AMA	Normal	46,XX	Mosaic +7	N/A	0.11	0.08
18	AMA, abnormal NIPS – 1p36 deletion	N/A	N/A	Mosaic +3 Normal [cultured cells]	N/A	0.27	0.18
19	Abnormal U/S – increased NT	Normal	46,XX	Mosaic multiple <i>de novo</i> CNVs on 8p Normal [cultured cells]	N/A	0.47/N/A [‡] /0.46	-0.39/N/A [‡] /-0.3
20	Abnormal maternal serum screen – T21	Normal [†]	47,XY,+10[8]/ 46,XY[30]	Mosaic +10 and mosaic +13 ^{¶¶}	N/A	0.23/0.71	0.16/0.44

CMA, chromosomal microarray analysis; CPM, confined placental mosaicism; AMA, advance maternal age; N/A, not available; U/S, ultrasound; NT, nuchal translucency; T21, trisomy 21; NIPS, non-invasive prenatal screening; CNV, copy number variation.

[†] The discordance between 13/18/21/X/Y FISH and CMA results may be explained by the different cell populations analyzed by these two different methods. Specifically, cells analyzed in Aneuploidy FISH were from the outermost cytotrophoblast layer given the short 5 minutes treatment time in trypsin according to the protocol in our laboratory. In contrast, CMA was conducted on DNA from intact CVS biopsy, which includes all

cytotrophoblast cells and the mesenchymal core. Therefore, the mosaic changes detected by CMA in chromosome 13 (Case 11 and 20) and in chromosome X and Y (Case 13) may not be present (or presented in a level that below the 10% reporting cutoff ratio) in the outermost cytotrophoblast layer in these cases, leading to a normal FISH result.

‡ Mosaic change not called by the in-house analysis program but noticed by visual inspection. See Figure 2 for CMA plots.

§ FISH analysis on cultured CVS cells of this sample showed no evidence of a copy number gain in chromosome 10.

¶¶ Only mosaic trisomy 13 detected by CMA on intact CVS sample of this case was interpreted as provisional CPM I but not the mosaic trisomy 10.

Table 3. Detailed information on the mosaic submicroscopic or nearly submicroscopic CNVs identified [†].

Case No.	Indication	CNV type	CNV Size (Mb)	Chromosome band(s)	Segmental duplication around breakpoint? [‡]	Recurrent or non-recurrent	Mosaicism type
4	Abnormal U/S – cystic hygroma	Mosaic loss	0.182	Xq27.3	No	Non-recurrent	CPM II or III
6	Abnormal U/S – increased NT	Mosaic loss	0.098	Xp22.31	No	Non-recurrent	CPM I
15	Abnormal maternal serum screen – T21	Mosaic gain	14.595	15q25.3q26.3	No	Non-recurrent	Provisional CPM I
16	AMA	Mosaic gain	1.333	10p12.33p12.31	No	Non-recurrent	Provisional CPM I
19 (CNV-1)	Abnormal U/S – increased NT	Mosaic loss	1.629	8p23.2	No	Non-recurrent	Provisional CPM I
19 (CNV-2)		Mosaic gain	0.781	8p23.2p23.1	No	Non-recurrent	
19 (CNV-3)		Mosaic gain	0.457	8p23.1	No	Non-recurrent	

CNV, copy number variation; U/S, ultrasound; AMA, advance maternal age; NT, nuchal translucency; CPM, confined placental mosaicism.

[†] Only CNVs are listed in this table; information on mosaic aneuploidies concurrently identified in Case 6 and Case 19 are listed in Table 1 and Table 2, respectively.

[‡] According to the Segmental Dup track from UCSC genome browser (hg19 build).

Figure 1. CMA plots from both CVS and AF sample types in confirmed CPM cases. A). Case 9: CMA whole genome plot from intact CVS (left) showing mosaic gain of chromosomes 11, 15 and 20, while plot from AF (right) shows no change. **B).** Case 4: CMA whole genome plot from intact CVS (left) showing mosaic loss at Xq27.3 involving the *FMR1* gene, while the plot from AF (right) shows no change. Enlarged CMA plots with arrows show the individual probe data for the region of the mosaic loss involving the *FMR1* gene. **C).** Case 6: CMA whole genome plot from intact CVS (top left) showing mosaic gain of chromosome 9 and the enlarged plot (bottom left) showing the individual probe data for the mosaic deletion of the *STS* gene.

The respective plots from AF (right) show neither change. D). Case 19: CMA chromosome 8 plot from intact CVS (left) showing multiple mosaic gains on chromosome 8p, while plot from AF (right) shows none of the changes.

Figure 2. Low level mosaic aneuploidies not called by the in-house algorithm, but identified through visual inspection. A). Case 13: CMA whole genome plot from intact CVS showing mosaic gain of chromosomes X and Y. B). Case 8: CMA whole genome plot from intact CVS (left) showing mosaic gain of chromosome 13, while plot from AF (right) shows no change.