Mosaicism for copy number variations in the placenta is even more difficult to interpret than mosaicism for whole chromosome aneuploidy

Lund, Ida C Bay; Becher, Naja; Graakjaer, Jesper; Lildballe, Dorte L; Uldbjerg, Niels; Bogaard, Pauline; Petersen, Astrid; Vestergaard, Else M; Vogel, Ida

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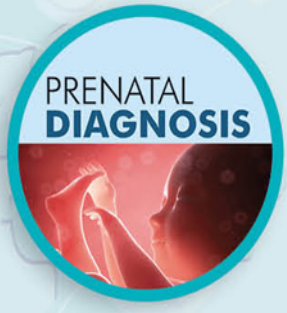

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Authors:
Ida Charlotte Bay Lund,[1,2,3] Naja Becher, [1,2,3] Jesper Graakjaer,[4] Dorte L. Lildballe,[2,4] Niels Uldbjerg,[5,6] Pauline Bogaard,[7] Astrid Petersen,[7] Else Marie Vestergaard,[1,2,8] Ida Vogel[1,2,6]

Affiliations:

[1]Department of Clinical Genetics, Aarhus University Hospital, 8200 Aarhus N, Denmark
[2]Center for Fetal Diagnostics, Aarhus University Hospital, 8200 Aarhus N, Denmark
[3]Department of Biomedicine, Aarhus University, 8000 Aarhus, Denmark
[4] Clinical Genetics, University Hospital of Southern Denmark, Sygehus Lillebaelt, 7100 Vejle, Denmark
[5]Department of Obstetrics and Gynaecology, Aarhus University Hospital, 8200 Aarhus N, Denmark
[6]Department of Clinical Medicine, Aarhus University, 8000 Aarhus, Denmark
[7]Department of Pathology, Aalborg University Hospital, 9000 Aalborg, Denmark
[8]Present address: Department of Clinical Biochemistry, Horsens Regional Hospital, 8700 Horsens, Denmark

Corresponding author:
Ida Charlotte Bay Lund
Aarhus University Hospital
Department of Clinical Genetics
Brenstrupgaardsvej 21 C,
8200 Aarhus N
Denmark
e-mail: idacharlottebaylund@dadlnet.dk
Phone: +45 2298 2281

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WHAT´S ALREADY KNOWN ABOUT THIS TOPIC?

- Mosaicism for Copy Number Variations (CNVs) of any size detected in chorionic villus samples (CVSs) may involve the fetus
- CNVs detected by amniocentesis may be discordant to aberrations detected in the postpartum placenta

WHAT DOES THIS STUDY ADDS?

- CVS mosaicism for CNVs may be unreliable in predicting fetal CNVs as such findings may differ from CNVs in the postpartum placenta. Counselling shouldn´t focus on the CNV region involved but on the option of amniocentesis.

Data availability statement: Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Abstract:

Objective: To compare mosaicisms in prenatal chorionic villus samples with corresponding postpartum placental samples.

Method: We collected placentas from 15 consecutive cases of mosaicism detected in chorionic villus samples and obtained five standardized samples on each placenta after delivery. All pre- and postnatal placental samples were uncultured and analyzed by high-resolution chromosomal microarray.

Results: Ten cases of mosaicism for whole chromosome aneuploidy(mWC) and five cases with mosaicism for (sub)chromosomal copy number variations(mCNVs) were included. In 5/10 mWC cases and in 4/5 mCNV cases the prenatally detected aberration was confirmed in the postpartum placenta. Three postpartum placentas revealed various complex aberrations differing from the prenatal results: 1) mosaicisms for different deletions/duplications on 9p and 9q in all samples (prenatal: mosaic 5.3 Mb duplication on 9p24), 2) different regions with deletions/duplications/loss of heterozygosity on 1p in all samples (prenatal: mosaic 2.3 Mb 1p36 duplication), and 3) mosaicism for a duplication on 5q and a deletion on 6p in one out of five samples (prenatal: mosaic trisomy 7).

Conclusion: CNVs constitute a complex subgroup in placental mosaicism. Counseling of these couples after chorionic villus sampling shouldn´t focus on the specific CNV involved, but on the nature of mosaicism and the option of amniocentesis and ultrasound.

Keywords: Microarray Analysis < FETAL GENETIC ANALYSIS, Array CGH < PRENATAL CYTOGENETICS, Chorionic villus sampling < FETAL MEDICINE and DIAGNOSTIC PROCEDURES, PRENATAL DIAGNOSIS

INTRODUCTION

In prenatal genetics, mosaicism detected after chorionic villus sampling is a recurring clinical challenge as discordance between the placenta and the fetus may exist. Mosaicism is defined as two or more distinct cell lines in the same individual arisen from one zygote[1]. Prenatal mosaicism is found in 1-2% of chorionic villus samples after conventional karyotyping of cultured cells and in 1-4% after chromosomal microarray on DNA extracted from uncultured cells [2-7]. The majority (80-90%) of these cases are classified as confined placental mosaicism (CPM) without fetal involvement [2; 6; 8]. Still, true fetal mosaicism (TFM) cannot be ruled out by a normal result on an amniocentesis and the risk of adverse outcome persists. Even without TFM, CPM may cause placental insufficiency leading to intrauterine growth restriction [9; 10].

Mosaicism in chorionic villus samples has been investigated thoroughly with conventional karyotyping[2; 7; 11]. However, only a few studies have primarily focused on evaluating mosaicism for Copy Number Variations (CNVs) of any size using chromosomal microarray[4-6]. The risk of TFM seems equally high in cases of mosaicism for whole chromosome aneuploidy and CNVs of any size, respectively [6]. In pregnancies affected by mosaicism for submicroscopic CNVs confined to the placenta, possible implications for the placental function have only been scarcely investigated [6; 12].

Previous studies comparing placental mosaicism pre- and postnatally were carried out in the 1980-90 ´s before the era of high resolution chromosomal microarray [9; 13-21]. We found no prior data on a consecutive case series of placental mosaicism comparing pre- and postnatal placental sample results using high resolution chromosomal microarray. Such studies are essential to understand and interpret fetoplacental mosaicism for any aberration detected after chorionic villus sampling in pregnancy. We present 15 consecutive cases of placental mosaicism in chorionic villus samples diagnosed by high resolution chromosomal microarray where follow-up with postpartum placental sampling has been performed. We report a new complexity to placental mosaicism with clinical implications related to interpretation of and counselling in cases of placental mosaicism.

METHODS

In the Danish prenatal screening program ~96% of all women participate in a combined 1st trimester screening. If further testing is indicated a total of 80% of women choose invasive testing over Non-Invasive Prenatal Testing and chorionic villus sampling is the more commonly used invasive method [22; 23]. According to the national prenatal guidelines, chromosomal microarray is applied on all chorionic villus samples regardless of indication [24; 25]. In our local Clinical Genetics Service, we receive approximately 600 chorionic villus samples annually and mosaicism is detected in overall ~4%.[6]

Ethical approval for the study was obtained by The Central Denmark Region Committee on Health Research Ethics (1-10-72-188-17). Pregnant women were consecutively recruited from the department of clinical genetics in Central Denmark Region from May 2017 to August 2018 if a transabdominal chorionic villus sampling had been performed and mosaicism for any aberration had been detected using chromosomal microarray. After approval from The Regional Research Ethics Committee Central Denmark Region (October 2017) we recruited pregnant women prospectively but we also searched our internal laboratory database for prenatal mosaic results to identify pregnant women who had received a mosaic result after CVS before October 2017 to boost the sample size. We included cases consecutively according to Supplementary Figure S1. When eligible participants had given consent to join the study the medical record were updated with information to the midwives to store the placenta after birth. On the consent form, participants could also permit data from the medical record to be used for the study. Abnormal outcome was defined as TFM, intrauterine death, stillbirth, preterm birth, intrauterine growth restriction or small-for-gestational-age infants (birth weight <-2SD).

From this point onwards, the abbreviation "CVS" is used for a chorionic villus sample obtained by chorionic villus sampling in pregnancy and "ppCVS" is used for a chorionic villus sample obtained by sampling the *p*ostpartum *p*lacenta.

Local standard procedures for prenatal chromosomal microarray on CVSs were followed in all cases. Briefly, DNA from 10-20mg uncultured and untryptinized CVS was analyzed with array-based Comparative Genomic Hybridization, aCGH (Sureprint G3 Human CGH microarray 180 K/ Agilent Technologies Inc., Santa Clara, CA, USA). CytoGenomics version 3.0.6.6 was used for data analysis. An average log2 ratio below the expected 0.585 for duplications and above -1.0 for deletions were evaluated for mosaicism. The level of mosaicism (frequency of

abnormal cells) (%) was determined based on the specific value of the log2 ratio by an experienced laboratory geneticist. The prenatally detected mosaic cases were categorized into two main groups: 1) mosaicism for whole chromosome aneuploidy, autosomal or sex chromosomal trisomies or sex chromosomal monosomy – as whole chromosome mosaicism (mWC) and 2) mosaicism for CNVs defined as any partial chromosome or submicroscopic aberration – as CNV mosaicism (mCNV).

Postpartum placentas were stored for a maximum of three days at +5°C or frozen at -18°C. Five ppCVSs were obtained from the fetal surface according to a standardized method where each ppCVS in all placentas was performed exactly the same way. The ppCVSs were obtained 1/3 radially from the placental margin at four quadrantal positions, clockwise, 12 (ppCVS_A), 3 (ppCVS_B), 6 (ppCVS_C) and 9 (ppCVS_D), and one in the middle (ppCVS_E) (Figure 1). Between each ppCVS new sterile instruments were used to avoid inter-sample contamination. The *pp*CVSs were stored at -80°C until dissection into 10 mg samples. The dissected samples were then washed in phosphate-buffered saline and treated with Proteinase K. DNA was isolated using the Matwell kit rack (Maxwell®16 MDx, Promega) and kept at -80 C until analyses. SNP arrays (Illumina CytoSNP-12 Single-Nucleotide-Polymorphism (SNP) -array; working resolution 50 kb) was applied on ppCVSs. DNA from the corresponding CVS was reanalyzed by SNP array for comparability as they were analyzed in the prenatal setting by aCGH. Genotypes in CVS and ppCVS from the same pregnancy were compared to rule out 100% maternal cell contamination in cases of normal female 46,XX. Genome Studio´s Heritability test was performed to check for sample switch and reduced data quality. KaryoStudio analysis software and BluefuseMulti were used for data analysis. Level of mosaicism was visually interpreted and estimated based on B-allele frequency according to Conlin et al. [26]. Results from each ppCVS were categorized as *fully concordant* (identical to the mosaicism detected in CVS, but the level of abnormal cell line was allowed to vary), *related concordant* (mosaicism at the same chromosome/chromosomal region as detected in CVS), *discordant abnormal* (mosaicism of a different chromosome than the mosaicism detected in CVS) or *normal* (normal non-mosaic molecular karyotype). These categories have been modified from Zhu et al. [27].

Study data were collected and managed using REDCap electronic data capture tools hosted at Aarhus University, Denmark. For statistical analysis we used Stata v.16. The Fisher´s exact test was used for comparison of proportions. The Danish Data Protection Agency approved the study (1-16-02-659-16). Six prenatal cases from the present study have been published

previously but postnatal results from postpartum placental samples were not included in this previous publication[6].

RESULTS

3.1 Overall comparison of pre- and post-natal chorionic villus samples

The 15 mosaic CVS results included ten cases of whole chromosome aneuploidy (mWC) and five cases with partial (sub)chromosomal aberrations (mCNV), Table 1. When we compared results from the ppCVSs and the prenatally obtained CVS, data were heterogenous. Individual results from each case are visually presented in Figure 1. SNP array plots of all aberrations are included in Supplementary Figure S2.

Regarding the ten cases of mWC, the exact aberration detected in the CVS was confirmed in the postpartum placenta in five cases (*fully concordant*) and not confirmed in five cases (*normal or discordant abnormal)*. One complex case with mosaicism for trisomy 7 in CVS (case 9), revealed different CNVs in one ppCVS; these CNVs had not been detected in the CVS (*discordant abnormal*).

Regarding the five cases with mCNV in the CVS, the aberration detected in the CVS was concordant (*fully or related concordant*) in four of the postpartum placentas. The aberrations detected in two of these placentas (cases 11 and 13) were, however, complex showing variation in near proximity to the region affected in the CVS (*related concordant*). In 1/5 of the mCNV-cases, no aberrations were found in the postpartum placentas (*normal*).

The probability of confirming mosaicism in the postpartum placenta was high in the CVSs with levels of mosaicism ≥30% (n=7) compared with the CVSs with a level of mosaicism <30% (n=8) (7/7, 100% vs 2/8, 25%, p=0.007). In general, when comparing levels of mosaicism between the CVS and the fully concordant ppCVSs within each case, differences were within a range of +/- 20%.

3.2 Complex results

Altogether, we had 3/15 particularly complex cases (cases 9, 11, 13) – all demonstrating mosaicisms for different CNVs in the postpartum placenta, which were not detected prenatally in the CVS. In these three placentas, the complex results between CVS and ppCVSs

could be categorized in *related concordant* (cases 11 and 13) or *discordant abnormal* (cases 11 and 9). All three cases had normal results on follow-up amniocentesis (aCGH) indicating CPM.

Figure 2 explains cases 11, 13 and 9 in detail and actual SNP array plots are included in Figure 3 and Supplementary Figure S2.

- Case 11:
  The CVS showed a mosaic 5.3 Mb 9p24 duplication. Postnatally, this specific duplication was not confirmed in any of the ppCVSs; instead, quite different aberrations located to 9p were detected in all five postpartum samples. These aberrations on 9p in the ppCVSs included mosaicism of deletions and duplications of various sizes and more than one aberration in the same ppCVS. All ppCVS_A-E results were different and ppCVS_E included additional aberrations on 9q. Furthermore, a mosaic 6.9 Mb duplication on chromosome 20p was detected in ppCVS_C.
- Case 13:
  The CVS showed a mosaic 2.3 Mb 1p36 duplication which was confirmed (*fully concordant*) in a mosaic state in ppCVS_E. The other four ppCVSs_A-D showed different aberrations on 1p; mosaicism of a deletion (ppCVS_A) and regions with loss of heterozygosity including a duplication (ppCVS_B-D).
- Case 9:
  Mosaicism for trisomy 7 was detected in the CVS but not confirmed in any of the ppCVSs (data not shown). Instead three aberrations, not detected in the CVS, were detected in ppCVS_D; mosaic 20 Mb duplication on 5q34, mosaic 7.2 Mb deletion on 6p25 and non-mosaic 762 Kb duplication on 8q23.

None of these three complex cases revealed, in any of the samples, a third haplotype in the B-allele frequency, indicating a Meiosis I error, in the SNP array results.

3.3 Clinical outcome

Overall, 5/15 pregnancies (33%, CI95%:13-61%) had an abnormal pregnancy outcome. Two pregnancies were classified as TFM by fetal buccal swab (case 1) or postnatal blood sampling (case 4). Three pregnancies affected by CPM had an adverse outcome (intrauterine death in case 10, intrauterine growth restriction in case 9 and small-for-gestational-age infant in case 6). The remaining ten pregnancies classified as CPM were uneventful and resulted in live born,

phenotypically normal infants.

Based on the results from three complex CPM cases (9, 11 and 13), where the ppCVSs were related concordantly or/and discordantly abnormal to the corresponding CVSs, we suggest a new sub-category of CPM termed *complex* CPM (Supplementary Figure S3).

DISCUSSION

In this study, we identify a new complexity to the field of placental mosaicism and show that mosaicism for CNVs detected prenatally by CMA can be accompanied by multiple aberrations, in the postpartum placenta such as deletions, duplications or/and loss of heterozygosity of varying size. This new knowledge is of outmost importance for clinical geneticists and fetal medicine experts and makes the interpretation of results, and the counseling of these patients, even more challenging. Adverse outcomes, such as TFM or CPM with stillbirth, intrauterine growth restriction or small-for-gestational-age infant, were overall frequent and found in 5/15 (33%, CI95%:13-61%) pregnancies although not among the five pregnancies with CPM for CNVs.

Placental whole chromosome mosaicism detected in a CVS, including evidence for the persistence of mosaicism to term, is reported in the literature based on conventional karyotyping and low-resolution chromosomal microarray [9; 13-21]. These previous studies have documented that mosaicism for whole chromosome aneuploidy or structural rearrangements detected in CVS have stabile cell lines; either the normal disomic and/or abnormal trisomic/monosomic/structural cell line detected is correspondingly detected in the postpartum placenta [9; 14-20]. This is in line with 9/10 cases of mosaic whole chromosome aneuploidy in our study where no other aberrations were detected in the postpartum placenta. We also found the same correlation as Kalousek et al.[9]; a level of mosaicism ≥30-35% in the CVS will increase the likelihood of confirmation in at least one postpartum placental sample. It may thus be suggested that the level of mosaicism in the CVS is correlated to the distribution of abnormal cell line(s) in the placenta.

The present case series highlights at least two important new findings in the postpartum placenta as compared to prenatal CVS; 1) complex variation in the near proximity to the chromosomal region affected in the CVS and 2) other aberrations including entirely different chromosomes. Such discrepancies between a mosaic CNV in a CVS and a mosaic CNV in the concordant postpartum placenta have, to our knowledge, not been reported before. This finding may potentially change the way we handle similar cases in the future – both in clinical genetics and in

fetal medicine.

Opstal *et al.* investigated postpartum placentas from pregnancies with discrepant results between Non-Invasive Prenatal Testing and CMA on amniotic fluid but they did not investigate CVSs [28]. Similarly, though, they detected different aberrations in the postpartum placenta not detected in the amniotic fluid. In the current study, two cases (11 and 13) were categorized in *related concordance* because different mosaic CNVs in the same chromosomal region as in the CVS were detected postpartum. Since both pregnancies had normal CMA result on the follow-up sample of uncultured amniocytes, the mCNV would likely be confined to the placenta. The most likely explanation for the confinement of these CNVs to the placenta is that these CNVs have originated due to mitotic errors during placental development. A single meiotic origin of these aberrations seems less likely because the CNVs could not be confirmed in the fetus. This hypothesis is supported by the B-allele frequency not displaying a third haplotype in any of the samples; although a Meiosis II error could not be excluded. Repair mechanisms in the following cell divisions may explain the occurrence of different CNVs or loss of heterozygosity in a specific chromosomal region. In the literature, a commonly accepted explanation of the mechanisms behind the origin of CNVs (mosaic or non-mosaic) has no definite evidence[29-31]. All three cases with mosaicism for smaller CNVs (<6 Mb) in CVS had *full* (case 7, 7q11 duplication) or *related mosaicism* (cases 11 and 13) distributed in all their individual ppCVSs. It could be speculated that there may be less selection pressure against smaller CNVs compared to mosaicism for an entire chromosome. Further investigations are needed before hypotheses can be generated for the mechanisms behind discrepancies involving the *same chromosome* in prenatal CVS and the corresponding ppCVSs.

The discovery of new aberrations involving entirely different chromosomes in the postpartum placenta, compared to the involved chromosome in the CVS, occurred in two cases (9 and 11). One explanation may be the nature of placental growth, which has been compared to tumor growth because it is invasive with a high mitotic index [32; 33]. Three of the "new" CNVs detected in the postpartum placental samples were large (20, 7.2 and 6.9 Mb respectively, Figure 2), and would likely be detectable by conventional karyotyping. The reason why such findings have not been reported before in postpartum placental studies using conventional karyotyping may be due to selection bias against cell lines with large CNVs during culturing. This would likely, if detected in one metaphase alone, have been interpreted as pseudomosaicism and disregarded [13]. Opstahl et al.[28] have detected mosaicism for a microscopic CNV with CMA, not detected by NIPT nor AC sample,

in one postpartum placental sample in one case. We saw this phenomenon of "new" CNVs not related to CVS results in only two postpartum placental samples. Thus, microscopically sized CNVs appear at specific placental sites and may be a rare event compared to an extensive load of small CNVs (<2Mb) detected in postpartum placentas in accordance with Kasak *et al.*[32]. It is possible, therefore, that CNVs of any size in the placenta may be missed in a CVS or CNVs which seem to have no clinical significance may be detected in a CVS. We do not know, however, if these CNVs develop over time ("in vivo culture" of the placenta) or if they are present at the gestational age where the chorionic villus sample is obtained. In the future, if resolution of Non-Invasive Prenatal Testing improves this subject can be further explored as cell-free placental DNA in maternal blood is believed to represent the entire trophoblastic layer of the placenta and not just a single site as a CVS[34].

Based on the findings discussed above, we suggest that it is redundant to complete a detailed evaluation when CNVs are detected in a mosaic state in uncultured cells from CVS by chromosomal microarray as other parts of the placenta, and potentially the fetus, may contain diverse but clinical significant CNVs. Counselling of couples after a diagnosis of mosaic CNV in a CVS is challenging and we may not need to focus on the specific chromosomal region involved. Instead, efforts should be put into understanding and explaining the nature of mosaicism and prepare the couple to cope with the long waiting time related to further investigations of these findings. If the couple wants to know more about the risk of TFM, amniocentesis should be offered. Fetal ultrasonography detecting malformations and growth retardation may be the modality to manage these cases until more knowledge regarding the mechanisms and clinical consequences of placental CNV mosaicism is obtained.

The possible implications of mosaic CNVs in the placenta on placental function need to be investigated further as our case-series is small. Outcomes in pregnancies affected by CPM for whole chromosome aneuploidy vary depending on the chromosome involved and the level and distribution of mosaicism [35]. We found no association between obstetric outcome and distribution of mosaic cell lines in the postpartum placenta. Contrary, Robinson et al. evaluated the frequency of trisomic cells by CGH (resolution >10 Mb) in postpartum placentas ascertained by intrauterine growth restriction and observed trisomic cell lines in 10% [36]. Likewise, pregnancies with abnormal outcome and normal (or no) CVS results could be due to placental mosaicism absent from the sampling site of the CVS; our results indicate that this may be a potential scenario. Postpartum placental studies with high resolution chromosomal microarray may be a supplement to placental

pathology examination in pregnancies of severe intrauterine growth restriction or fetal death, if fetal investigations do not provide an explanation.

One limitation of this study is that culturing of the pre- and postnatal samples was not performed. Therefore, we could not supplement our results with conventional karyotyping or FISH, but next generation cytogenetics after whole genome sequencing could be applied for a better understanding of the somatic chromosomal variation in the placenta in future studies investigating uncultured samples. Furthermore, we cannot discuss whether the mosaicism was detected in cytotrophoblasts or in mesenchymal cells but there is always a risk of TFM regardless of which chorionic villi layer is affected by an abnormal cell line[2].

CONCLUSION

Mosaicism for CNVs detected in uncultured CVSs constitute a complex subgroup and seems unreliable for the prediction of fetal phenotype and placental function. Regardless of affected CNV region in mosaic state, these results should be followed by amniocentesis and detailed fetal ultrasonography if the couple wants to know more about the risk of TFM. Repair mechanisms after prenatal de novo CNVs are evident and need further exploration to understand the nature of the placenta in more detail. Data on amniocentesis, fetal/newborn tissue and long-term follow-up of live born infants should be systematically collected for a large international cohort of cases of mosaicism. Such data could be used to increase knowledge in this field making also mosaic results in a CVS contribute with reliable and useful information. This may also provide new insight into unknown repair mechanisms of CNVs.

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Table 1. Prenatal chorionic villus sample results and clinical characteristics from the 15 included cases.

Type of mosaicism	Given case nb	Aberration in CVS sample by CMA during pregnancy	1. trimester characteristics					Birth characteristics				Placental characteristics		
			Indi-cation (GA at CVS)	MA (y)	PaPP-A (MoM)	ß-hCG (MoM)	NT mm	Outcome	GA at birth	BW (g)	BW (z-score)	PW g (percentile)	Diameter mm (range)	Sample W median mg (range)
Common aneuploidy, n=3														
	1	T21	NT> 3.5 mm (12+5)	27	0.482	0.465	3.8	TOP	14+4	na	na	na	55-90	197 (100;281)
	2	T13	cFTS> 1:100 (13+2)	35	0.548	0.980	2.6	LB	39+6	4175	1.3	634 (>90th)	210-220	288 (232;499)
	3	T13	TR (FHR: 130) (13+3)	34	0.675	1.064	2.1	LB	40+1	4370	1.6	570 (50th)	170-190	371 (336;492)
Sex chromosomal aberrations, n=2														
	4	X (XX)	Family history (10+4)	35	0.445	2.349	1.4	LB	40+5	3540	-0.2	457 (~10th)	170-195	185 (135;247)
	5	X(XX)	cFTS> 1:300 (13+3)	32	0.472	2.938	2.0	LB	41+5	3760	-0.1	na	160-180	224 (164;355)
Other whole chromosome aneuploidy, n=5														
	6	T7	cFTS> 1:100 (13+1)	32	0.212	0.901	1.5	LB	38+6	2855	-2.2	368 (<10 th)	140-150	223 (135;296)
	7	T3/T7/T15	Family history and cFTS 1:700-1:300 (12+6)	43	7.089	1.968	2.0	LB	38+1	4265	2.6	790 (>90th[†])	na	na
	8	T11	NT> 3.5 mm (13+4)	44	2.499	0.999	3.9	LB	41+3	3140	-1.6	419 (<10th)	na	na
	9	T7	Family history (13+1)	37	0.476	0.705	2.8	LB	37+2	2100	-2.5	340 (<10th[‡])	na	na
	10	T7	cFTS> 1:300	30	0.657	0.521	3.0	IUD[§]	36+3	3180	0.8	646 (>90th)	na	na

			(14+2)											
CNV/partial chromosome aberration, n=5														
	11	9p24.1p23 dup (5.3 Mb)	cFTS> 1:300 (11+6)	36	0.316	0.362	1.2	LB	40+1	3590	-0.2	740 (na†)	170-200	448 (277;533)
	12	T18p or tetrasomy 18p	cFTS> 1:300 (13+0)	37	0.267	0.232	0.8	LB	40+0	3000	-1.5	355 (<10th)	165-180	361 (301;481)
	13	1p36.33 p36.32 dup (2.3 Mb)	PaPP-A<0.2 MoM (13+2)	28	0.199	0.399	2.5	LB	38+0	2498	-1.9	335 (<10th)	140-145	273 (172;403)
	14	7q11.21 dup (1.5 Mb)	cFTS> 1:100 (13+1)	32	0.401	1.654	2.3	LB	40+4	3328	-0.7	540 (na†)	170-180	275 (208;334)
	15	8q13.2q24. 3 dup (75 Mb)	NT≥ 3.5 mm (14+0)	31	1.090	0.864	3.5	LB	39+4	3706	0.6	640 (na†)	190-190	na

† Placenta weighed fresh

‡ Maternal antithrombin deficiency and previous child with intrauterine growth restriction

§ Significant heart malformation detected at gestational week 29. IUD in gestational week 36.

Placentas were either stored cold (n=10; maximum of three days) or frozen at -20C (n=3; maximum of 13 days). Two placentas were accidentally fixed in formalin (case 8 and 10) before samples. Macro- and microscopically pathology examination of placentas did not reveal any certain characteristics for mosaicism except in case 10 where oedema of larger villi was found; this finding could be due to mosaicism but also fetal hydrops.

Abbreviations: ß-hCG, free ß human chorion gonadotropin; BW, birth weight; cFTS, combined first trimester screening; CMA, chromosomal microarray; CVS, chrorionic villus sampling; ; dup, duplication; FHR, fetal heart rate; GA, gestational age; IUD, intrauterine death; LB, liveborn and apparently healthy at birth; MA, maternal age; MoM, multiple of the median; na, none available; NT, nuchal translucency measurement; PaPP-A, pregnancy associated protein A; pp, postpartum placental; PW, placenta weight, TOP, termination of pregnancy; TR, tricuspid regurgitation.

Figure Legend

Figure 1. Results from postpartum chorionic villus samples in 15 placentas.

The fetal site of the postpartum placenta is shown for all 15 cases. Each postpartum chorionic villus sample is marked by a circle.

FIGURE 2 Legend

Figure 2. Three placentas with complex results

The figure shows four complex results from three placentas: Case 11 (2A and 2C), Case 13 (2B) and Case 9 (2D). In Figure 2A, 2B and 2C the six bars display the results from the prenatal chorionic villus sample (CVS) and the five postpartum chorionic villus samples (ppCVS_A-E). In Figure 2D, three bars show results from a single ppCVS_D and three bars show joined normal results from CVS and ppCVS_A-C,E. Deletions are coloured in red and duplications are coloured in blue. The intensity of the colours matches the level of mosaicism. Loss of heterozygosity (LOH) is coloured in yellow. SNP plots can be found in Figure 3 and Supplementary Figure S2.

2A:
Results from chromosome 9 of Case 11 are shown.
CVS: 5.3 Mb dup, arr[GRCh]9p24.1p23(8065133-13415908)x~2-3 (Level mos 55-60/60%).
ppCVS_A: 16.3 Mb del, (arr[GRCh37]9p24.3p22.2(1019207- 17340864) x~1-2 (Level mos 75-80%) and a 4.2 Mb dup, (arr[GRCh37]9p22.2-21.3(17720175- 21920346) x~2-3) (Level mos 70-80%)
ppCVS_B: Non-mosaic 285 Kb dup (arr[GRCh37]9p24.3(46587-331490)x3)
ppCVS_C: 6.4 Mb del, (arr[GRCh37]9p24.3-24.1(1501977-7902733) x~1-2) (Level mos 80%). and a 1.1 Mb dup (arr[GRCh37]9p24.1-p23(7,945,146-9,064,330) x~2-3) (Level mos 50%)
ppCVS_D: 9.8 Mb dup (arr[GRCh37]9p24.3-p23(271132-10112766) x~2-3) (Level mos 60%)
ppCVS_E: 2.8 Mb del, arr[GRCh37]9p24.3-p24.2(46587-2851026) x~1-2 and a 3.4 Mb dup (arr[GRCh37]9p24.2-p24.1(2871439-6272766) x~2-3). On 9q, 52 Mb del ((arr[GRCh37]9q21.129q33.2(72291978-124650611)x ~1-2) and terminal higher mosaic level of 0.6 Mb del arr[GRCh37]9q34.3(139999888-139403102 x ~1-2)). Mosaic level in sample E cannot be validly estimated due to maternal cell contamination.

2B:

Results from chromosome 1p of Case 13 are shown.
CVS: 2.3 Mb dup (aCGH: arr[GRCh37]1p36.33p36.32(564424-2842457)x~2-3 ; SNP-array: arr[GRCh37]1p36.33p36.32(752566-2817421)x~2-3) (Level mos 40-50/50-55%)
ppCVS_A : (MCC): 7.6 Mb mosaic deletion (MCC<15%) was detected (arr[GRCh37]1p36.33-p36.23(752566-8304607)x~1-2)
ppCVS_B : LOH of 31 Mb (arr[GRCh37]1p36.33-p35.2(752566-31713684)x2 hmz) (Level mos na) and a 1.8 Mb non-mosaic dup with LOH in the middle of the dup (arr[GRCh37]1p35.2-p35.1(31719450-33515210)x3)
ppCVS_C: LOH of 1.9 Mb (arr[GRCh37]1p36.33-p36.32(753541-1146459)x2 mos hmz) (Level mos na but lower than in sample D)
ppCVS_D: LOH of 1.9 Mb (arr[GRCh37]1p36.33-p36.32(753541-1146459)x2 mos hmz) ppCVS_E: 2.3 Mb dup (arr[GRCh37]1p36.33p36.32(1113121-2883858)x~2-3) (Level mos 50-55%).

2C:
Results from chromosome 20 of Case 11 are shown.
CVS, ppCVS_A,B,D,E: normal
ppCVS_C: 6.9 Mb dup (arr[GRCh37]20p13-p12.3(63,244-6,970,514) x~2-3.

2D:
Results from chromosome 5, 6 and 8 from Case 9 are shown.
CVS: Mosaicism for Trisomy 7 (data not shown)
ppCVS_D: 20 Mb dup on 5q (arr[GRCh37]5q34-q35.3(160069545-180705539)x~2-3), 7.2 Mb del on 6p (arr[GRCh37]6p25.3-p24.3(204909-7374207)x~1-2) and 762 Kb dup on 8q (arr[GRCh37]8q23.3p24.3(115543245-116305599)x3). LOH on chromosome 8 is not shown because all samples from case 9 showed LOH on random chromosomes due to consanguineous parents (8.8% consanguinity).

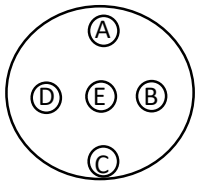
Abbreviations: CVS, chorionic villus sampling; dup, duplication; del, deletion; Level mos, level of mosaicism (frequency of abnormal cell line)

Figure 3. SNP array plots of Case 11 and Case 13 showing related concordant results between chorionic villus sample and postpartum chorionic villus samples at sites A-E.

Case 11: The six plots show the different aberrations (deletions and duplications) on chromosome 9 from the different samples.

Case 13: The six plots show the different aberrations (deletions, duplication and loss of heterozygozity) on chromosome 1p from the different samples. The duplication on CVS and ppCVS_E is exactly the same.

Abbreviations: CVS, chorionic villus sample; ppCVS, postpartum chorionic villus sample; MCC, maternal cell contamination.



Normal karyotype ⁎MCC in biopsy

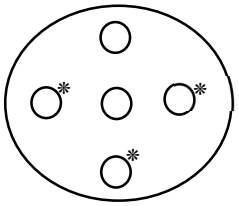
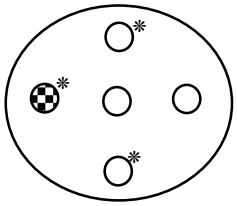
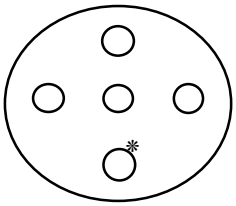
Mosaic result (full concordance to CVS)

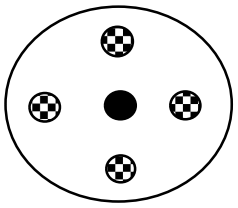
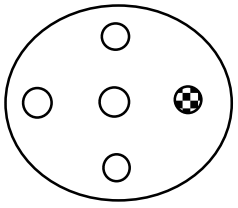
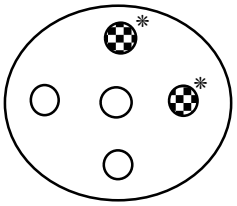
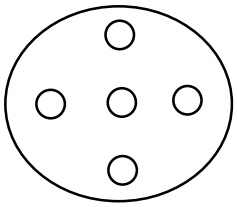
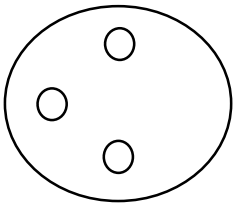
Non-mosaic abnormal result (full concordance to abnormal cell line in CVS)

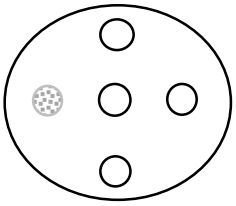
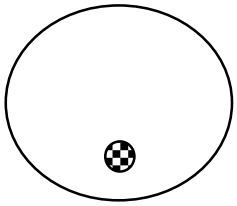
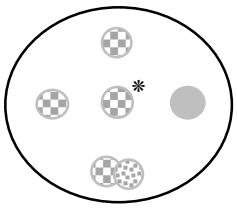
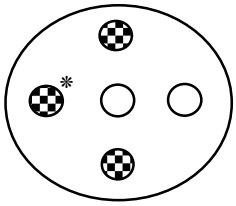
Mosaic result (related concordance to CVS)

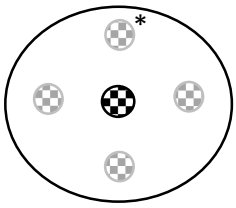
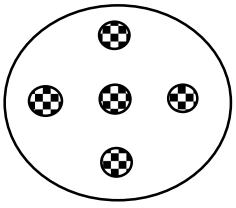
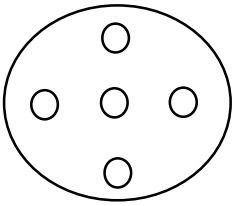
Non-mosaic abnormal result (related concordance to CVS)

Mosaic result (discordance to CVS – mosaicism including a different chromosome than the CVS result)

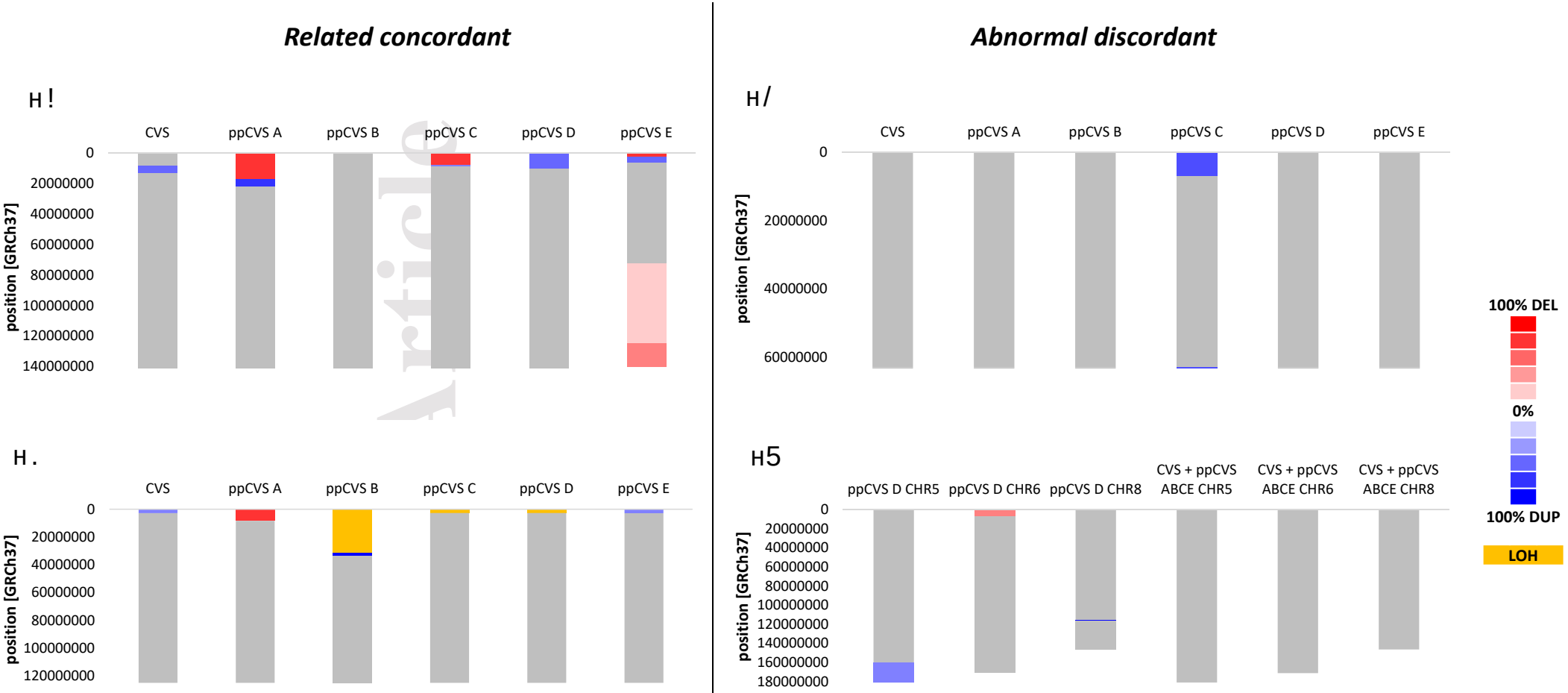
Given Case number	CVS aCGH - Karyotype (arr [GRCh37])	ppCVSs	Amniocytes – aCGH - Karyotype (GRCh37)	Postnatal Genetic analyses
1	(21)x2~3 20-30% T21			Buccal swab: T21 100% T21
2	(13)x2~3 10-20% T13	 ppCVS_D: 70-75%	(1-22,X)x2	na
3	(13)x2~3 15-25% T13		(1-22)x2, (X,Y)x1	na

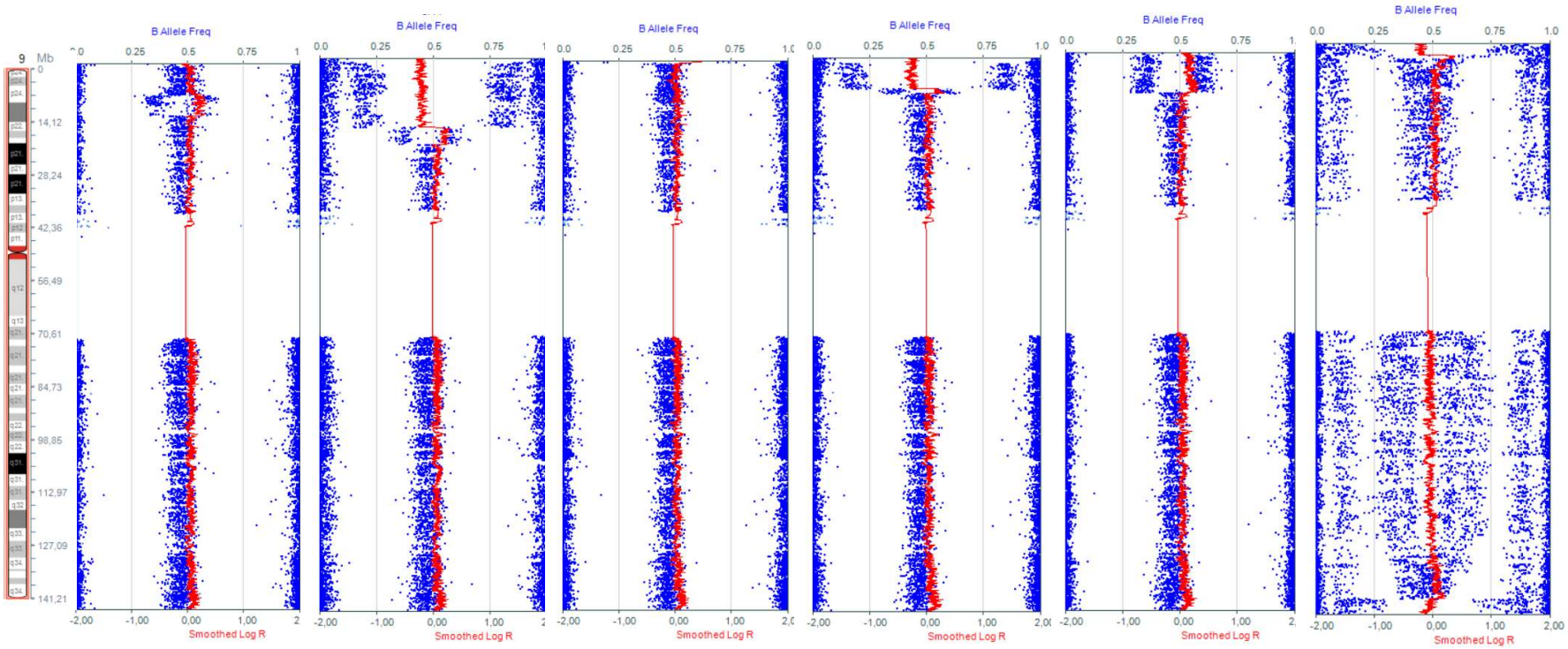
4	(X)x1~2 80-90% Monosomy X	 ppCVS_A: 85-90% ppCVS_B: 90-95% ppCVS_C-D:>95%	na	Postnatal blood: mos 45X[1]/46,XX[9]. ish mos Xcen(DXZ1x1)[8]/ishX cen(DZX1x2)[42] 15%
5	(X)x1~2 50% Monosomy X	 ppCVS_B: 85-90%	(1-22,X)x2	na
6	(7)x2~3 55-65% T7	 Level of mosaicism not possible due to MCC	(1-22,X)x2	na -
7	(3)x2~3, (7)x2~3, (15)x2~3 <10% T3+T7+T15		(1-22)x2, (X,Y)x1	na -
8	(11)x2~3 <10% T11	 ppCVS B and E: Failed (Formalin fixed)	(1-22)x2, (X,Y)x1	na -

9	(7)x2~3 10-15% T7	 See Figure 2†	(1-22,X)x2	na	
10	(7)x2~3 55-65% T7	 ppCVS_C: 65-70% ppCVS_A-B and d-E: Failed (Formalin fixed)	(1-22)x2, (X,Y)x1	Pericardium: (1-22)x2, (X,Y)x1 by aCGH	-
11	9p24.1p23 (8065133- 13415908) x2~3 dn‡ 55-60% 9p24 dup (5.3 Mb)	 See Figure 2 and 3 ppCVS_C: one sample but two categories of results	(1-22)x2, (X,Y)x1	na	
12	(18p)x2~4 25-30% T18p or 10-15% Tetrasomy 18p		(1-22)x2, (X,Y)x1	na	

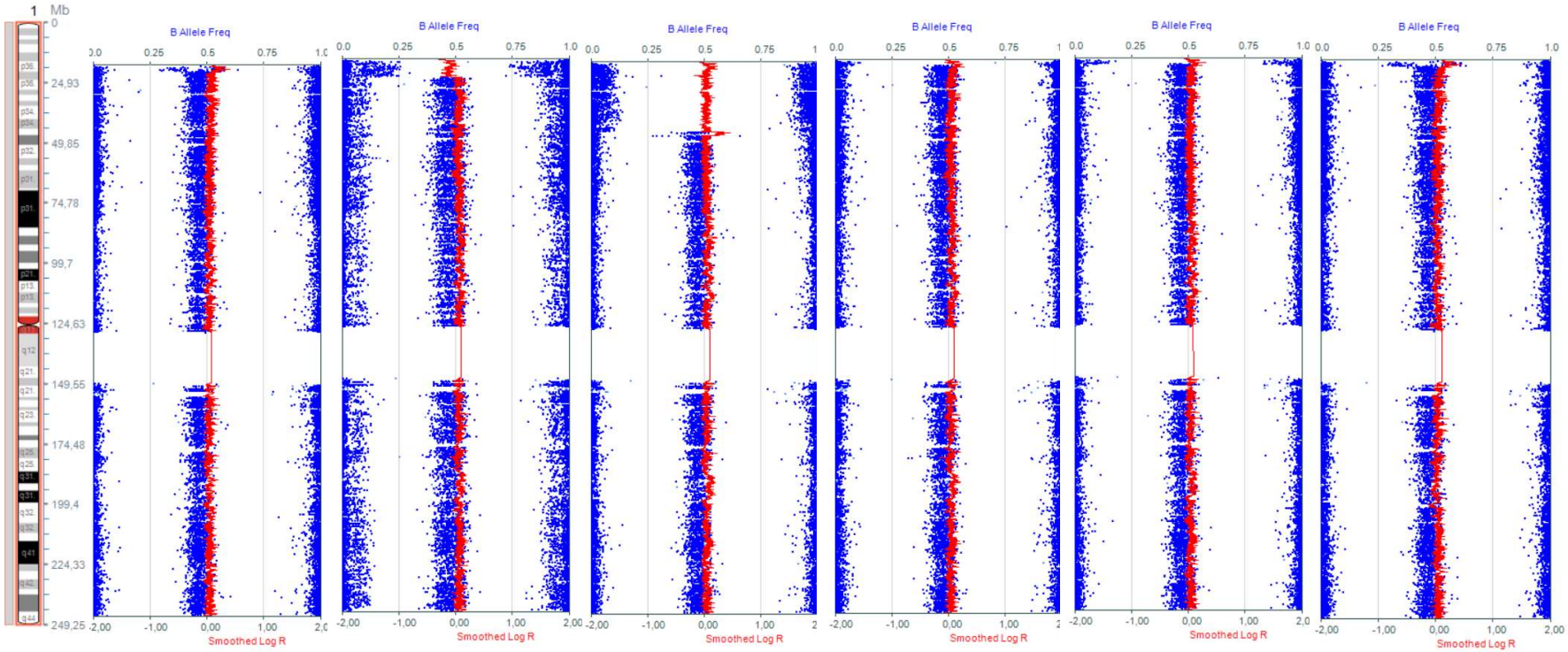
13	1p36.33 p36.32 (564424-2842457) x2~3 40-50% 1p36 dup (2.2 Mb)	 See Figure 2 and 3	(1-22)x2, (X,Y)x1	na
14	7q11.21 (62833583-64767333) x2~3 50% 7q11 dup (1.9 Mb)	 65-70% in all ppCVSs	na -	
15	8q13.2q24.3 (69836784-145129457) x2~3 20-30% 8q13 dup (75 Mb)		(1-22,X)x2	

All abnormal ppCVS results can be found in Supplementary Figure S2. Results from repeated CVS analyses by SNP-array have not been included as these were concordant to aCGH results (max +/-20% difference in level of mosaicism); except two cases (case 8 and 9) where SNP-array did not confirm the prenatally detected low-level mosaicism by aCGH. This may reflect differences in sensitivity for detection of mosaic abnormalities of the two methods.
†Regions with loss of heterozygosity on many chromosomes due to consanguinity (not on chr. 7)
‡Parental array karyotypes were normal
Abbreviations: CVS, chorionic villus sample; dup, duplication; MCC, maternal cell contamination; ppCVS, postpartum CVS





M



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