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CLINICAL REPORT

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Confined placental mosaicism involving multiple de novo copy number variants associated with fetal growth restriction: A case report

Giulia F. Del Gobbo[1,2] | Victor Yuan[1,2] | Wendy P. Robinson[1,2]

[1]BC Children's Hospital Research Institute, Vancouver, Canada

[2]Department of Medical Genetics, University of British Columbia, Vancouver, Canada

Correspondence
Wendy P. Robinson, BC Children's Hospital Research Institute, 950 W 28th Ave, Vancouver, Canada, V5Z 4H4.
Email: wrobinson@bcchr.ca

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Abstract

The presence of multiple large (>1 Mb) copy number variants (CNVs) in non-malignant tissue is rare in human genetics. We present a liveborn male with a birth weight below the first percentile associated with placental mosaicism involving eight 2.4–3.9 Mb de novo duplications. We found that the duplications likely co-localized to the same cells, were mosaic in the placenta, and impacted maternal and paternal chromosomes. In addition, 27.4 Mb and 240 genes were duplicated in affected cells, including candidate placental genes *KISS1* and *REN*. We ruled out involvement of homologous recombination-based mechanisms or an altered epigenome in generating the CNVs. This case highlights the diversity of genetic abnormalities in the human placenta and the gaps in our knowledge of how such errors arise.

KEYWORDS
CNV, de novo, fetal growth restriction, mosaicism, placenta

1 | INTRODUCTION

Copy number variants (CNVs) are an important source of genetic variation in humans. The majority of CNVs are small; only about 3% of healthy adults carry a large rare CNV >1 Mb (Collins et al., 2020). This rate is higher in populations with congenital abnormalities, developmental delay, or neurodevelopmental disorders (Girirajan et al., 2011). The occurrence of several large rare CNVs in one individual is extremely rare even in clinical populations. Large chromosomal aberrations are common in early development (van Echten-Arends et al., 2011), however, abnormal embryos are typically not viable unless mosaicism with a normal cell population occurs and the abnormal cells are mainly restricted to extraembryonic tissues (Lestou & Kalousek, 1998). This confined placental mosaicism (CPM) may impact placental function and lead to poor pregnancy outcomes like fetal growth restriction (FGR) (Lestou & Kalousek, 1998). We report a novel case of CPM involving eight 2.4–3.9 Mb de novo duplications associated with FGR. We explore the potential of these CNVs to explain FGR and possible mechanisms of origin.

2 | MATERIALS AND METHODS

Ethics approval was obtained from the University of British Columbia/Children's and Women's Health Centre of B.C. Research Ethics board (H17-01545). The case (PM324) was identified from a cohort of placentas from control and small-for-gestational age (SGA; birth weight <10th percentile) pregnancies profiled for CNVs using the Infinium Omni2.5–8 BeadChip array (Illumina, San Diego, USA)(Del Gobbo et al., 2021). Due to case deidentification, minimal clinical data were available. The case was ascertained due to a prenatal diagnosis of symmetric FGR of unknown cause (Lausman et al., 2013). The mother

was of normal BMI and did not smoke. A male infant was born at 40 weeks gestation with a birth weight of 2600 g (<1st percentile, adjusted for sex and gestational age; Kramer et al., 2001). The course of the pregnancy was otherwise normal. The placenta was <3rd percentile in weight, and histological exam showed mildly immature villi for the gestational age, but was otherwise unremarkable.

Samples of chorionic villi (vil), amnion, and chorion were obtained from four distinct placental cotelydons (sites 1–4), in addition to umbilical cord. Part of each sample of chorionic villi was enzymatically digested to produce samples enriched for the trophoblast and mesenchyme of the villi (Robinson et al., 2010). In addition to two samples (vil1, vil4) previously analyzed (Del Gobbo et al., 2021), DNA from vil2 and vil3 was screened for CNVs using the Omni2.5–8 array (Del Gobbo et al., 2021). Genotyping of microsatellite loci within the duplicated regions in all available tissues was used to confirm array findings, determine parental origin, and assess level of mosaicism (Robinson et al., 2010) (Appendix S1; Table S1). DNA from maternal blood was used to assess maternal genotype.

Imprinted genes, placental imprinted differentially methylated regions (DMRs), and genes with elevated placental expression were identified as previously described (Del Gobbo et al., 2021). Coordinates of segmental duplications and repeat DNA were accessed from the genomicSuperDups and RepeatMasker tables from the UCSC Browser; fragile sites from the HumCFS database (Kumar et al., 2019); and placental partially methylated domains (PMDs), blocks of low-methylated DNA characteristic of the placental epigenome, as previously described (Yuan et al., 2021). Enrichment of elements near breakpoints was assessed by permutation tests using the *regioneR* package in R, with 10,000 permutations selecting random non-overlapping regions of the same size in the genome.

To determine potential alterations in DNA methylation (DNAme), DNA from vil1 and vil4 were assessed on the Infinium MethylationEPIC BeadChip (Illumina), along with chorionic villus samples from 19 healthy term pregnancies. Data were processed as described (Yuan et al., 2021) and methylation beta (β) values were extracted for DNAme analysis.

3 | RESULTS

We previously identified eight 2.4–3.9 Mb interstitial duplications in seven chromosomes in a placental chorionic villus sample (vil1) (Del Gobbo et al., 2021) (Table 1). Microarray assessment of three additional samples from the placenta (vil2-vil4; Figure 1(a)) suggested absence of these or other large CNVs. Microsatellite genotyping of all extraembryonic samples confirmed that the proportion of cells containing each independent duplication was similar (Table S2), therefore, we concluded that they were likely de novo and co-occurred in the same cells. Averaging estimates across all loci tested indicated that vil1 had ~60% abnormal cells, with the trophoblast more affected than the sample enriched for mesenchyme (72% and 22%, respectively; Figure 1(b)). Additionally, low levels of abnormal cells (<10%) were estimated in site 3, near to site 1 (Figure 1(a), (b)). The amnion and umbilical cord, most similar in developmental origin to fetal tissues, were unaffected, suggesting that the duplications were likely confined to the placenta (Figure 1(b)). One duplication involved the maternal chromosome, four involved paternal chromosomes, and three were uninformative for parental origin (Table 1).

Among the eight CNVs, >27.4 Mb was duplicated (Table 1). The CNVs were absent from population controls (Collins et al., 2020; MacDonald et al., 2014), and did not overlap known microduplication syndrome loci. One pathogenic and seven likely pathogenic duplications overlapped four of the CNVs (1q32.1, 5q35.1, 7q11.21q11.22, 11p11.2) by at least 50% (Table S3)(Firth et al., 2009; Landrum et al., 2018; Olson et al., 2012). Of the associated cases, only one, with a likely pathogenic 1.17 Mb duplication in 5q35.1, showed evidence of poor growth (Table S3). In total, 240 genes were involved in the duplications (Table 1), 40 of which were disease-associated in OMIM, and several are highly expressed in placenta (*KISS1*, *REN*, *LARGE2*, *MNTR1B*, and *VSTM5*). One duplication overlapped placental-specific imprinted DMRs near *PRDM11* and *MAPK8IP1*.

To explain the simultaneous occurrence of eight duplications, we searched for features that might be enriched around (<100 kb) the 16 CNV breakpoints. These were not associated with chromosome fragile sites, early- or late-replicating regions, or placental PMDs ($p > 0.05$). There were no pairs of segmental duplications near CNV breakpoints, nor was there enrichment of segmental duplications or Alu, LINE-1, or LTR repetitive elements ($p > 0.05$).

To explore whether an unusual epigenetic profile may have contributed to genomic instability or impacted placental function, we compared DNAme in vil1 (containing CNVs) to vil4 (balanced) and 19 term controls. DNAme in vil1 was not distinct based on genome-wide principal components analysis, sample pairwise correlations,

TABLE 1 Eight large duplications present in a mosaic state in case PM324 placenta

Genomic coordinates (hg19)	Cytogenetic band	Size (Mb)	Parental chromosome	Genes (N)	Genes of interest
Chr1:200,478,352-204,413,297	1q32.1	3.93	Maternal	71	*KISS1*, *REN*, *KDM5B*
Chr5: 169,133,115-172,752,205	5q35.1	3.62	Paternal	31	
Chr6: 66,855,754-69,301,518	6q12	2.45	Unknown	0	
Chr7: 65,791,671-69,249,095	7q11.21-q11.22	3.46	Unknown	15	
Chr8: 92,757,374-96,311,905	8q21.3-q22.1	3.55	Unknown	29	
Chr11: 43,851,111-47,385,923	11p11.2	3.53	Paternal	53	*LARGE2*
Chr11: 90,310,352-93,636,999	11q14.3-q21	3.33	Paternal	16	*MTNR1B*, *VSTM5*, *PRDM11*, *MAPK8IP1*
Chr17: 48,475,076-52,011,849	17q21.33-q22	3.54	Paternal	25	

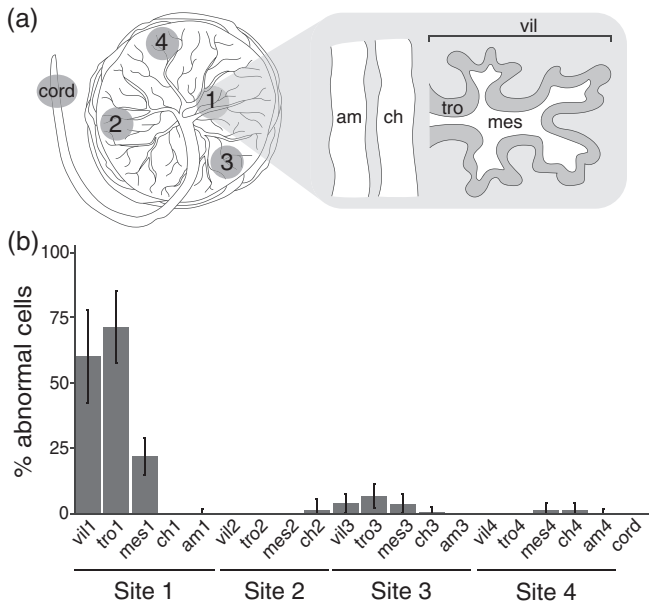

FIGURE 1 Estimated percentage of cells carrying the eight duplications in available samples from PM324 placenta and associated fetal membranes. (a) Schematic of tissues sampled, including chorionic villi (vil), enzymatically separated trophoblast (tro) and mesenchyme (mes) from villi, chorion (ch), and amnion (am) from four distinct locations in the placenta (sites 1–4), and umbilical cord. Circles are not to scale. (b) Mean percentage of abnormal cells in each sample calculated from all informative microsatellite loci tested within the duplications. Error bars indicate *SD*

overall methylation β-value distribution, nor DNAme of PMDs (Figure S1). On average, DNAme in the duplications tended to be lower in vil1 compared to vil4 and term controls (Figure S1c-d; Table S4).

4 | DISCUSSION

We describe the first example of multiple large (>1 Mb) de novo duplications identified in the placenta from an infant with FGR. The duplications were mosaic, impacted localized regions of the placenta, and involved both parental chromosomes, indicating a post-zygotic origin. As levels were highest in trophoblast, and because enzymatically separated mesenchyme retains up to 50% trophoblast cells (Yuan et al., 2021), we presume the duplications are confined to the trophoblast. Additionally, the consistency of the level of mosaicism among duplications within individual samples suggests that they arose simultaneously in one cell early in development.

Chorionic villus trees grow clonally from a few precursors shortly after implantation (Castellucci et al., 1990; Peñaherrera et al., 2012). Because abnormal cells were present in two separate sampling sites, representing two different cotelydons, but absent from others, the mutational event most likely occurred in a trophectoderm cell after blastocyst formation but prior to primary villus formation. The apparent patchy distribution of mosaicism is expected given the placental tree structure, and does not allow inference of any selective growth advantage/disadvantage of the abnormal cells.

The duplications may have impacted placental function and thereby fetal growth, as some relevant genes were duplicated, including *KISS1*, involved in trophoblast migration and angiogenesis and over-expressed in preeclampsia (Bilban et al., 2004; Francis et al., 2014; Zhang et al., 2011), and *REN*, dysregulated in preeclampsia and involved in trophoblast proliferation (Lumbers et al., 2019). One paternal duplication involved polymorphic, maternal-imprinted placental DMRs associated with *PRDM11* and *MAPK8IP1* (Hanna et al., 2016), and one duplication overlapped a likely pathogenic CNV in a patient with poor growth. Despite these lines of evidence, much of the placenta was chromosomally normal, therefore, it remains possible that other unidentified factors contributed to the severity of FGR in this case.

The cause of this unusual multi-CNV event is unclear. Lack of evidence for large homologous sequences around CNV breakpoints argues against homologous recombination-based mechanisms. DNAme in vil1 containing the duplications was unaltered, although this does not exclude that epigenetic defects early in development may have been involved, as we tested placental tissue after birth. Due to limited microarray probe density, we could not determine exact coordinates of the CNV breakpoints to perform sequence analysis to identify signatures of non-homologous, replication-based mechanisms of CNV origin.

The occurrence of eight large duplications of consistent size is nonetheless remarkable, and there are few similar reports. Chromoanagenesis may generate multiple large CNVs, however, the limited number and dispersal of the present duplications across several chromosomes does not fit with known molecular features of chromoanagenesis (Zepeda-Mendoza & Morton, 2019). Recently, the presence of 4–9 de novo CNVs, mainly duplications >100 kb, was reported in 5 of 60,000 individuals from a clinical population (Liu et al., 2017). These multiple de novo CNVs were associated with replication-based mechanisms, evidenced by short microhomologies and microhomeologies near breakpoints, and mosaicism was not observed (Liu et al., 2017). Another case of an SGA infant was reported with a placenta carrying 3 "partial trisomies": a 22 Mb dup(6)(p22.3pter), a 5.8 Mb dup(9)(q34.13), and a 22 Mb dup(21)(q21.2qter), present in only one of five placenta biopsies (Zamani Esteki et al., 2019). The alterations were all terminal, in contrast to the smaller interstitial duplications we identified.

This case is unique and relevant to the study of the diversity of genomic abnormalities in humans. Because mosaic abnormalities may persist in the placenta even when the fetus is normal, abnormalities such as this one, although rare, may be more prevalent in placental tissues. For example, this case was found among 54 SGA placentas screened for CNVs (Del Gobbo et al., 2021). Future studies profiling CNVs and other genomic alterations in the placenta should consider testing multiple distinct regions to further explore such mosaicism. With increasing use of non-invasive testing to detect fetal genomic abnormalities from placental DNA in maternal blood, it is important to understand the diversity of genomic abnormalities in the placenta,

how often they may be confined to extraembryonic tissues, and their incidence in normal and uncomplicated pregnancies.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Wendy P. Robinson https://orcid.org/0000-0002-2010-6174

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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