Genomic Imbalances in the Placenta Contribute to Poor Fetal Growth

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Abstract

Background: Fetal growth restriction (FGR) is associated with increased risks for complications before, during, and after birth, in addition to risk of disease through to adulthood. Although placental insufficiency, failure to supply the fetus with adequate nutrients, underlies most cases of FGR, its causes are diverse and not fully understood. One of the few diagnosable causes of placental insufficiency in ongoing pregnancies is the presence of large chromosomal imbalances such as trisomy confined to the placenta; however, the impact of smaller copy number variants (CNVs) has not yet been adequately addressed. In this study, we comprehensively evaluate the contribution of both placental aneuploidy and CNVs to fetal growth.

Methods: We used molecular-cytogenetic approaches to identify aneuploidy in placentas from N=101 infants born small-for-gestational age (SGA), typically used as a surrogate for FGR, and from N=173 non-SGA controls from uncomplicated pregnancies. We confirmed aneuploidies and assessed mosaicism by microsatellite genotyping. We then profiled CNVs using high-resolution microarrays in a subset of N=53 SGA and N=61 control euploid placentas, and compared the load, impact, gene enrichment and clinical relevance of CNVs between groups. Candidate CNVs were confirmed using quantitative PCR.

Results: Aneuploidy was over 10-fold more frequent in SGA-associated placentas compared to controls (11.9% *vs.* 1.1%; p=0.0002, OR=11.4), was confined to the placenta, and typically involved autosomes, whereas only sex chromosome abnormalities were observed in controls. We found no significant difference in CNV load or number of placental-expressed or imprinted genes in CNVs between SGA and controls, however, a rare and likely clinically-relevant germline CNV was identified in 5.7% of SGA cases. These CNVs involved candidate genes *INHBB*, *HSD11B2*, *CTCF*, and *CSMD3*.

Conclusions: We conclude that placental genomic imbalances at the cytogenetic and submicroscopic level may underlie up to ~18% of SGA cases in our population. This work contributes to the understanding of the underlying causes of placental insufficiency and FGR, which is important for counselling and prediction of long term outcomes for affected cases.

Background

Fetal growth restriction (FGR), where the fetus does not grow to its genetic potential, affects 5–12% of pregnancies in developed countries (1). FGR is associated with increased risk for perinatal, neonatal, pediatric and long-term adult health complications (2–6). Often small-for-gestational-age (SGA, birth weight < 10th percentile) is used as a surrogate for FGR, however, a subset of SGA infants may be small but normally grown for their potential and thus otherwise healthy. In particular, pathologically growth-restricted infants are at increased risk for morbidity and mortality.

Poor growth *in utero* is most commonly attributed to placental insufficiency, however fetal infection or genetic abnormality, and maternal health or lifestyle factors may also play a role (7–9). Some of these factors (i.e. maternal smoking, infection, obesity) may also contribute to poor trophoblast development

and function, thus the etiologies of FGR and placental insufficiency are complex and intertwined. A major known cause of placental insufficiency in a viable pregnancy is confined placental mosaicism (CPM), where some or most cells in the placenta are aneuploid, while the fetus has a predominantly normal diploid chromosome complement. CPM identified prenatally is associated with increased risk for FGR and other pregnancy complications depending on the levels of abnormal cells and the chromosome(s) involved (10, 11). Screening placentas postnatally has also confirmed a contribution of CPM to FGR (12–15). We previously identified trisomy CPM in 4/43 FGR pregnancies, but none in 85 controls nor 18 cases associated with preeclampsia (PE) without FGR (16). Despite the evidence that large genomic imbalances in the placenta are associated with FGR, few studies have investigated the role of smaller genetic imbalances (< 5–10 Mb), copy number variants (CNVs). To date, studies investigating CNVs associated with FGR have either not studied placental tissue (17, 18) or had small sample sizes and found conflicting results (19, 20).

In this study, we aimed to thoroughly evaluate the contribution of placental genomic imbalances to poor fetal growth. To this end, we assessed i) the incidence of large aneuploidies (> 15 Mb) in 274 placentas from control and SGA pregnancies, and ii) the load, impact, and clinical relevance of placental CNVs (< 15 Mb) to SGA in a subset of 114 euploid placentas. This is the largest study to date of its kind; it enhances our understanding of the underlying causes of placental dysfunction and poor fetal growth, and further establishes the importance of assessment of CPM in the clinic.

Methods

Research ethics approval

Ethics approval for use of human research subjects in this study was obtained from the University of British Columbia/Children's and Women's Health Centre of British Columbia Research Ethics board (H17-01545) and from the Hospital for Sick Children (1000038847) and Mount Sinai Hospital (05-0038-E) Research Ethics boards. Informed written consent was obtained from all study participants.

Sample collection and cohort characteristics

Vancouver Cohort

Placental samples for the Vancouver cohort were ascertained and processed as described[19] and include cases used in previous studies (16, 21–24). Clinical information, including newborn sex and birth weight, gestational age at delivery, maternal age, and ethnicity were collected. Placental and maternal samples were processed and DNA was extracted as previously described(16).

This cohort (N = 207) included 136 controls from uncomplicated pregnancies (no SGA, hypertension/PE, or known abnormal maternal serum screen results) and 71 cases of SGA (Table 1). Exclusion criteria

were a prenatally-diagnosed chromosome abnormality or congenital anomaly in the fetus. SGA was defined as birth weight < 10th percentile, adjusted for sex and gestational age at birth based on Canadian growth charts (25). The majority, 55/71 (77%) of SGA cases met criteria for FGR, defined as birth weight < 3rd percentile, or < 10th percentile with additional findings suggestive of placental insufficiency, including i) persistent uterine artery notching at 22–25 weeks, ii) absent or reversed end diastolic velocity on umbilical artery Doppler, and/or iii) oligohydramnios (amniotic fluid index < 50 mm). One FGR case had a birth weight > 10th percentile but was diagnosed as FGR from prenatal measurements and severe oligohydramnios. Preeclampsia (PE) was defined according to Canadian criteria(26) as previously described(24). Following aneuploidy assessment, euploid placentas from a subset of N = 24 control and N = 29 SGA cases, 90% of which fulfilled criteria for FGR, were selected for further CNV profiling (Table 1). These were randomly selected after excluding cases and controls associated with a twin pregnancy (N = 23), or known maternal smoking during pregnancy (N = 3). Figure 1 summarizes the study design and number of cases per cohort used at each analysis step.

Table 1
Study cohort clinical characteristics

Group	Gestational age at birth (w), mean (range)	Maternal age at birth (y), mean (range)	Sex, N male (%)	Birthweight (S.D.), mean (range)	Twins, N (%)	PE, N (%)
Vancouver cohort - Total samples						
Control (N = 136)	39.2 (30.1–41.9)	34.3 (23.8–45.8)	68 (50)	0.1 (-1.2-2.7)	11 (8)	0 (0)
SGA (N = 71)	35.3 (23.6–41.7)*	35.2 (23.1–41.0)	34 (48)	-1.9 (-3.6- -1.2)*	12 (17)	31 (44)
Subset of samples for CNV profiling						
Control (N = 24)	39.3 (38.0-41.4)	34.8 (30.2–40.5)	13 (54)	0.01 (-1.1-2.2)	0 (0)	0 (0)
SGA (N = 29)	34.9 (24.0-40.6)*	34.4 (23.9–42.9)	18 (62)	-1.9 (-3.0- -0.6)*	0 (0)	11 (38)
Toronto cohort – Total samples						
Control (N = 37)	37.1 (27.3–41.0)	32.9 (21–43)	19 (51)	0.28 (-1.1-1.5)	0 (0)	N/A
SGA (N = 30)	34.0 (27.1–38.6)*	35.1 (25–44)	9 (30)	-2.2 (-3.5-1.2)*	5 (17)*	N/A

*$p < 0.05$, *p*-values calculated in comparison to respective control groups by Student's t-test for maternal age and birth weight, Mann-Whitney U-test for gestational age, and Fisher's exact test for all categorical variables. SGA, small-for-gestational age; PE, preeclampsia; N/A, not available.

Toronto cohort

The Toronto cohort was ascertained and processed as part of a distinct study, and findings from the two cohorts were then subsequently compared. Placental samples were obtained as previously described (27). Clinical information including newborn sex, birth weight, and gestational age were collected for all cases. The original cohort included N = 99 samples, however following microarray quality filtering, N = 67 remained, including placentas from 37 control and 30 SGA pregnancies (Table 1, Fig. 1). Definitions for control and SGA followed the same criteria as the Vancouver cohort, described above. Exclusion criteria were a prenatally-diagnosed chromosome abnormality or congenital anomaly in the fetus, CMV or toxoplasmosis infection, or clinical amnionitis. Additionally, cases or controls were excluded if mothers

were diagnosed with: i) preconceptional severe hypertension; ii) clinically significant thrombophilia; iii) advanced renal, heart or liver failure; iv) type I diabetes mellitus or gestational diabetes requiring treatment with insulin; or v) anemia and autoimmune disorders requiring therapy during pregnancy.

Aneuploidy screening and CPM follow-up

Aneuploidy was detected using several methods in this study. In the Vancouver cohort, samples were assessed by comparative genomic hybridization (CGH), which can detect aneuploidies greater than 15 Mb, or by multiplexed ligation-dependent probe amplification (MLPA) of subtelomeric probes (SALSA MLPA Subtelomeres Mix, MRC-Holland, NL), designed to detect aneuploidies that extend to the ends of the chromosome (Fig. 1). A subset of these samples (N = 85 control and N = 43 SGA), all screened by CGH, have been previously published (16); the current study is a retrospective re-assessment of aneuploidy in those cases, with additional samples collected. For more recent cases, MLPA was used to screen for aneuploidy because it is a reliable and cost-effective method to identify whole chromosome aneuploidies (monosomy and trisomy), as well as terminal duplications and deletions. In the Toronto cohort, aneuploidy was detected using CNV profiling by microarray (see below). All cases with an aneuploidy detected by any method was further assessed by microsatellite polymorphism genotyping of probes on the involved chromosome (**Additional File 1: Supplementary Methods**). Aneuploidies identified by MLPA were also confirmed using CNV profiling by microarray to determine the extent of the alteration, particularly in cases where results suggested abnormalities restricted to one chromosome arm (see below, **Supplementary Methods**).

Microarray processing and CNV detection

Placental DNA was assessed on the Infinium Omni2.5-8 BeadChip array (Illumina, USA) for the Vancouver cohort, and on the Affymetrix CytoScan HD array (ThermoFisher Scientific, USA) for the Toronto cohort (Fig. 1) at The Centre for Applied Genomics, Toronto, Canada (28, 29). In the Vancouver cohort, an additional DNA sample from a different location in each placenta was also run on the array to assess the possibility of detecting mosaicism of CNVs by high-density microarray (**Supplementary Methods**). Following sample quality checks unique to each array type, all 54 Vancouver cases and 67/99 Toronto cases were available for analysis (Fig. 1). CNVs were detected using in-house pipelines (28, 29) applying 3–4 CNV-calling algorithms specific to each array platform (**Supplementary Methods**). Following CNV quality checks, high-confidence CNVs called by at least two algorithms with a minimum 50% reciprocal overlap, ≥ 5 probes, and ≥ 10 kb were kept for analysis. CNV boundaries were compared to the Database of Genomic Variants and in-house databases of CNVs in controls, and rare CNVs were defined as those present in < 0.1% of controls and at least 50% unique. Given discordance in CNV calls between technical replicates of placental DNA (**Supplementary Methods**, **Supplementary Fig. 1**), mosaicism of CNVs was not investigated and the DNA sample with the higher microarray quality scores from each placenta was selected for CNV analysis for the Vancouver cohort. Ancestry was assessed using SNP genotypes by

MDS clustering of identity-by-state distances in PLINK (30) (**Supplementary Methods**). The ancestry composition of both cohorts was comparable (**Supplementary Table 1, Supplementary Fig. 2**).

Candidate CNVs

CNVs with potential clinical relevance to SGA were prioritized based on: whether they were rare, ≥ 200 kb, overlap pathogenic or likely pathogenic CNVs in the DECIPHER or ClinVar databases, overlap genes with important roles in placental function or those that are reported to be differentially expressed or with variants associated with growth restriction. CNVs were categorized following American College of Medical Genetics guidelines (31). Candidate CNVs were confirmed and assessed for CPM using quantitative PCR (**Supplementary Methods**).

Placental-enhanced and imprinted genes

A list of 356 genes with elevated expression in the placenta was downloaded from the Human Protein Atlas (32), including 78 with placental-specific elevated expression. A database of imprinted regions was curated from the OTAGO Imprinted Genes (33) and GeneImprint (34) databases, and reported placental imprinted differentially methylated regions (DMRs)(35, 36) (**Table S2**). Outer genomic boundaries were used to generate a consensus region for those genes associated with a placental imprinted DMR.

Functional pathway enrichment

Enrichment of 2,191 GO and KEGG (37) pathways in genes with coding sequences impacted by rare CNVs in SGA was assessed using a generalized linear model with universal gene count correction in the *cnvGSA* R package. Sex and cohort (array) were included as covariates, and thresholds of 100-1,500 genes were used to limit pathways assessed. A false-discovery rate (FDR) of < 0.1 was used to define significantly enriched (coefficient > 0) or deficient (coefficient < 0) pathways in SGA CNVs.

Statistical analyses

Continuous variables were compared using the Student's t-test or Mann-Whitney U test depending on whether the data was normally-distributed by the Shapiro-Wilk normality test. Categorical variables were compared by Fisher's exact test. Bonferroni correction for multiple testing was used where applicable. Statistical power for comparing CNV load was assessed using the *pwr* package in R. Based on a previous report of a large effect size ($d > 0.95$) in the difference in CNV load in control *vs.* SGA placentas (19), we assumed a slightly lower but still large effect size (d) of 0.8. Based on the minimum sample size in each group per cohort (N = 24) and using an $\alpha = 0.05$, our study had > 80% power to detect significant differences in each cohort individually. Analyses were performed in R version 3.5.1 (38), and plots were generated using the *ggplot2*, *ggbio*, and *ggpubr* packages.

Results

Poor fetal growth is associated with placental aneuploidy

Aneuploidy screening was performed in 207 placentas from the Vancouver cohort and 67 placentas passing microarray quality checks from the Toronto cohort. Amongst 173 control placentas, no cases of CPM or autosomal aneuploidy were detected. Two (1.1%) controls had constitutional abnormalities involving the sex chromosomes (Table 2), one of which only impacted Yqter. In contrast, amongst 101 SGA cases, 12 (11.9%) had a whole or partial autosomal trisomy present in the placenta (Table 2) (p = 0.00017, OR = 11.4; Fisher's exact test). These were found both in cases of isolated SGA and SGA in association with maternal PE.

Table 2
Summary of findings from detection of placental aneuploidy.

Study Group (N)	Balanced (M:F)	Unbalanced (M:F)	CGH/MLPA result	Inferred karyotype	CPM
Control (173)	171 (86:85)	2 (1:1)	Gain of X	47, XXX[]	No
			del(X/Yq)	46,XY,del(Yqter)	Unk.
SGA (101)	89 (39:50)	12 (4:8)	Gain of 7	47,XX,+7/46,XX[]	Yes
			Gain of 7	47,XY,+7/46,XY[]	Yes
			Gain of 2	47,XX,+2/46,XX[]	Yes
			Gain of 13	47,XX,+13/46,XX[]	Yes
			dup(7q),del(Xp)	46,XX,der(X) t(X;7) (p22.2;q21.2)/46,XX	Yes
			del(4q),dup(4p)	46,XY, der(4)del(4)(q34.2), dup(4) (p16.3p15.31)/46,XY	Yes
			Gain of 10, Gain of X	48,XXX,+10/47,XXX	Yes[‡]
			N/A	47,XY,+2/46,XY	Unk.
			N/A	46,XX,+i(15q)/46,XX	Unk.
			N/A	47,XX,+16/46,XX	Yes
			N/A	47,XX,+16/46,XX	Yes
			N/A	47,XY,+16/46,XY	Yes

[]Cases published in Robinson *et al*. 2010 (16); [‡]Constitutional trisomy X, CPM of trisomy 10. CGH, comparative genomic hybridization; MLPA, multiplexed ligation-dependent probe amplification; CPM, confined placental mosaicism; Unk., unknown/unable to confirm; N/A, not available (cases were only screened by microarray).

Of the cases with successful follow-up (10/12), all abnormalities in SGA placentas were determined to be CPM based on microsatellite genotyping (Table 2). Four of these cases were previously published[19], however 8 are new and confirm that CPM is a significant cause of SGA. Of the 9 cases with available maternal DNA, uniparental disomy (UPD) was excluded in the diploid cell population from all but one previously-published case with CPM for trisomy 2 and probable upd(2)mat (16). The incidence of aneuploidy did not differ between cohorts (2/136 *vs.* 0/37 controls and 7/71 *vs.* 5/30 SGA in the Vancouver and Toronto cohorts, respectively). Overall, our cohorts had high maternal ages (Table 1), and among the SGA cases, maternal age tended to be higher in pregnancies associated with CPM than those

without a placental aneuploidy (**Supplementary Table 3**), though this was not significant ($p > 0.05$, Student's t-test).

Load of CNVs does not differ between SGA and control placentas

To explore the role of placental CNVs in *in utero* growth, 114 euploid placentas from control and SGA newborns were assessed using high-density microarrays (Fig. 1). We found one SGA case (PM324) with mosaicism for 8 large 2–4 Mb duplications in the placenta (**Supplementary Fig. 3**). As the combined level of aneuploidy exceeded 27 Mb, it was an outlier that was excluded it from further comparisons, so as to not bias results; we instead considered it as an additional case of placental segmental aneuploidy. Due to significant differences in load of CNVs between the different array platforms (**Supplementary Table 4**), we performed case-control comparisons within each cohort independently. We found no difference in total number and cumulative extent (bp) of CNVs per placenta, except for a greater cumulative bp of rare CNVs in SGA placentas in the Vancouver cohort ($p = 0.03$, Mann-Whitney U test) (Table 3). When comparing these measures by gains and losses separately, there were also no significant differences (Table 3).

Table 3
Summary of load of CNVs in control and SGA placentas.

	Vancouver Cohort		Toronto Cohort	
	Control (N = 24)	**SGA (N = 28)**	**Control (N = 37)**	**SGA (N = 25)**
N CNVs	**17 (11–25)**	**16 (9–27)**	**35 (20–57)**	**32 (22–52)**
Gains	7 (1–11)	7 (1–14)	17 (9–38)	15 (8–36)
Losses	10 (4–20)	9 (3–17)	18 (9–33)	17 (10–29)
N rare CNVs	**4 (1–10)**	**4 (2–10)**	**7 (1–29)**	**6 (1–19)**
Gains	1 (0–6)	2 (0–6)	4 (0–22)	3 (0–16)
Losses	3 (0–7)	3 (0–9)	3 (0–12)	3 (0–6)
Cumul. size (Mb)	**1.22 (0.44–3.43)**	**1.59 (0.57–5.12)**	**3.20 (1.25–7.86)**	**3.17 (1.63–5.80)**
Gains	0.57 (0.03–1.32)	0.86 (0.03–2.85)	2.36 (1.06–6.97)	2.05 (0.97–5.11)
Losses	0.65 (0.06–2.92)	0.72 (0.11–3.45)	0.84 (0.18–2.33)	1.12 (0.36–4.19)
Cumul. size rare (kb)	**219 (10–902)**	**327 (74–864)***	**893 (38 – 3,652)**	**825 (19 – 3,329)**
Gains	100 (0-781)	197 (0-819)	678 (0–3,460)	516 (0–2,937)
Losses	119 (0-485)	130 (0-517)	1,570 (0–5,776)	1,373 (0–4,516)

 excludes outlier PM324. *$p < 0.05$, Mann-Whitney U-test. All values reported as mean (range). Cumul., cumulative

As larger CNVs are more likely to be impactful, we compared CNV size across all placentas in each group. In the Vancouver cohort, CNVs were larger in SGA placentas ($p = 0.002$, Mann-Whitney U test; **Supplementary Fig. 4**). When considering CNV gains and losses separately, only the losses were significantly larger ($p = 0.010$, Mann-Whitney U test; **Supplementary Fig. 4**). When separated by sex, the larger CNV sizes in SGA were significant only amongst females (**Supplementary Fig. 5**). There were no significant differences between groups in the Toronto cohort.

To further assess whether SGA placentas had a greater CNV load, we compared the number of gains or losses per placenta at size bins ranging from < 15 kb to > 3 Mb in all CNVs or only in rare CNVs between groups. There were no consistent differences between SGA and control placentas. SGA placentas in the Vancouver cohort had fewer small losses (< 15 kb, $p = 0.002$; Mann-Whitney U-test), and those in the Toronto cohort had more large losses (500 kb-1 Mb, $p = 0.001$; Mann-Whitney U-test). Both of these findings withstood multiple test corrections at a Bonferroni-corrected p-value threshold of $p = 0.005$, but were not observed in rare CNVs (Fig. 2).

Candidate CNVs identified in SGA placentas

We next focused on rare CNVs ≥ 200 kb as these are most likely to contribute to the SGA phenotype. There were 34 large rare CNVs present in SGA placentas and 53 in controls. CNVs with potential roles in placental function and/or fetal growth were identified 5.7% (3/53) of SGA placentas but not in controls (0/61). The 3 candidate CNVs were categorized as variants of uncertain significance (VUS)-likely pathogenic and impact the functionally relevant genes *IHNBB, HSD11B2*, *CTCF*, and *CSMD3* (Table 4). These were confirmed by qPCR to be present in both placenta and cord blood, thus were not confined to the placenta.

Table 4
Candidate CNVs with clinical relevance to SGA identified in study placentas.

Case ID	Sex	Study group	Genomic coordinates (hg19)	Size (kb)	CNV type	Genes	Category	CPM
7665	Female	SGA	2:121,092,278 – 121,914,455	822	Gain	*INHBB*, *GLI2*	VUS-likely pathogenic	No
6234	Female	SGA	16:67,150,183 – 67,615,830	466	Loss	*HSD11B2*, *CTCF*, 21 others	VUS-likely pathogenic	No
10506	Female	SGA	8:112,947,262 – 116,124,691	3,177	Loss	*CSMD3*	VUS-likely pathogenic	No

CPM, confined placental mosaicism; VUS, variant of uncertain significance

No difference in total, placental-enhanced, or imprinted genes impacted by placental CNVs

To investigate potential impact of CNVs, we compared the number of genes involved in CNVs per case. We found no differences in the Vancouver cohort, however there was a trend for a greater number of genes affected by losses in SGA placentas in the Toronto cohort ($p = 0.049$, Mann-Whitney U-test; Fig. 3). There were no significant differences when focusing on rare CNVs.

We did not find an enrichment of genes with enhanced placental expression in SGA CNVs, however there were more losses of placental-enhanced genes in controls in the Toronto cohort ($p = 0.02$, Fisher's exact test; **Supplementary Table 5**) that was not reproduced in the Vancouver cohort. Gains impacting *ERVV-1* and *ERVV-2*, and CNVs impacting several *PSG* family genes, a region known to be copy number variable in the human population(39), were common in both cases and controls.

We did not find any significant enrichment of imprinted regions in placental CNVs from SGA cases (**Supplementary Table 6**). Several common CNVs impacting imprinted regions were recurrent, including

placental imprinted DMRs for *SPRN* and *CYP2E1* (**Supplementary Table 7**). CNVs deemed as rare were also recurrent, including gains impacting *KCNK9* and the DMR near *PRMT2* (**Supplementary Table 7**). One rare CNV was present uniquely in a SGA case, arr[hg19] 22q11.21(19,931,668 – 19,980,300)x1, overlapping the placental-specific imprinted DMR and coding sequence of *ARVCF*. One other CNV resulted in a deletion of the growth-related gene *INS* in a control: arr[hg19] 11q15.5(2,170,670-2,199,458)x1 (**Supplementary Table 7**).

No significantly enriched gene pathways in SGA placentas

Out of 1,872 GO and KEGG pathways with genes involved in rare CNVs, we did not find any significantly enriched pathways in SGA CNVs (FDR > 0.4). 8 pathways were enriched at a nominal $p < 0.05$, the top being “negative regulation of cell cycle” ($p = 0.031$), and 7 were deficient (**Supplementary Table 8**). Investigating gains and losses separately, no enriched pathways were identified (FDR > 0.4). 10 pathways were enriched in SGA gains at a nominal $p < 0.05$, the top being “regulation of cellular response to stress” ($p = 0.009$), and three pathways were deficient in SGA gains (**Supplementary Table 8**).

Discussion

In this study, we investigated the contribution of genomic imbalances in the placenta to poor fetal growth. In our otherwise low-risk population, we found that CPM involving trisomy or large segmental aneuploidy was present in 11.9% of SGA cases, or 12.7% when including the case with duplications totaling > 27 Mb. The significant contribution of trisomy CPM to SGA/FGR confirms previous reports (12–16), however we have additionally identified cases of CPM of large segmental aneuploidies contributing to SGA, including a dup(7)(q21.2q36.3), del(X)(p22.2) likely deriving from a X;7 translocation event, and a case with dup(4)(p16.3p15.31), del(4)(q34.2). Although CPM can occur in healthy pregnancies (14, 40–42), only non-mosaic aneuploidies affecting the sex chromosomes were identified in our controls.

While the incidence of placental aneuploidy associated with SGA in this study is comparable to past reports (13, 15, 16), it is expected to be population-dependent. The frequency of trisomy, and thus CPM, increases with advanced maternal age, which is also a risk factor for SGA. Indeed, we found that maternal age tended to be higher in SGA pregnancies with CPM (mean: 36.7 y) than those that were chromosomally-balanced (mean: 35.0 y). Conversely, CPM should contribute to fewer cases of SGA in populations with high rates of other risk factors for SGA, such as maternal smoking or poor nutrition (43, 44). A higher CPM incidence is also expected using a stricter definition of FGR rather than SGA, e.g. fetal weight < 3rd percentile or by using biomarkers like placental growth factor (PlGF) in maternal serum that are predictive of placental-mediated FGR (45). Although we could not measure maternal PlGF levels, our SGA group was likely enriched for cases of pathological growth restriction as a large proportion of cases were < 3rd percentile (68% Toronto cohort, 48% Vancouver cohort) and the majority of cases in the Vancouver cohort met criteria for FGR.

Overall, we could not confirm previous reports finding decreased (19) or increased (20) load of CNVs in SGA placentas compared to controls. Small sample size may explain these discrepancies, as both past studies had < 10 cases per group. With greater sample size and low incidence of other risk factors in our population, we were well poised to detect genetic contributors to SGA. Although we identified trends that suggest that some SGA placentas have an increased load of large CNVs, our findings did not support that placental CNVs commonly contribute to SGA. We also did not find significant differences in number of total or placental-expressed genes or imprinted regions in CNVs, which also suggests that either these are not major drivers of poor fetal growth in our cohort or their effects are subtler than we had power to detect.

Nonetheless, a candidate VUS-likely pathogenic germline CNV was identified in 5.7% of SGA placentas in this study. This incidence is similar to past studies of prenatal samples, which identified pathogenic CNVs in 3–7% of cases of isolated FGR with normal karyotypes (17, 18, 46). Case 7665 has a duplication of *INHBB*, which encodes a subunit for the activin and inhibin proteins that play important roles in trophoblast growth and invasion (47, 48), and altered mRNA or protein levels of these compounds are associated with miscarriage, severe PE, and FGR (49). Case 6234 has a deletion encompassing *HSD11B2* and part of *CTCF*. *HSD11B2* is highly expressed in placental trophoblast cells, and encodes 11β-HSD2, which regulates fetal exposure to maternal glucocorticoids (50). Reduced placental gene expression or protein levels has been associated with FGR (51–54), and patients with rare mutations in *HSD11B2* have significantly lower birth weight (55). *CTCF* is a highly-conserved transcription factor, and rare loss-of-function variants or deletions of the gene are associated with low birth weight, postnatal growth retardation, microcephaly and intellectual disability (56). Case 10506 had a 3 Mb deletion encompassing *CSMD3*, which is reported to be intolerant to loss-of-function variants (upper bound o/e = 0.3 in gnomAD (57)), and *Csmd3* knockout mice display lower body length and body fat (58).

Strengths and limitations

This is the first study to our knowledge to characterize both aneuploidy and copy number variants in the placenta in association with poor fetal growth. It also contributes the largest sample evaluated for the association between placental CNVs and SGA to date, incorporating rigorous data processing using well-established pipelines, and several thorough lines of investigation. Due to the retrospective nature of this study, differences exist in clinical characteristics and methodologies between the cohorts and are a limitation of the study. Certain exclusion criteria used in the Toronto cohort were not available in the Vancouver cohort (e.g. infection during pregnancy), therefore we could not exclude such cases. Additionally, the aneuploidy screening methods used were not fully equivalent, as MLPA cannot detect large interstitial duplications or deletions. Despite this, the Vancouver and Toronto cohorts had similar clinical characteristics (Table 1, **Supplementary Table 1**) and the methods to screen for aneuploidy all accurately identify whole chromosome or chromosome arm abnormalities, therefore we combined the cohorts to improve our power to establish the contribution of placental aneuploidy to SGA. We were unable to combine the two cohorts to study CNV load associated with SGA due to the significant

differences between the high-density microarrays used for CNV detection. However even when assessed separately, each cohort had adequate power to identify differences at the large effect sizes described in previous reports (19, 20), and testing the two cohorts independently gave us the opportunity to assess the reproducibility of our findings.

Research and clinical implications

An appreciation for the impact of placental aneuploidy on SGA/FGR is relevant for both research and clinical applications. For studies investigating the etiology of idiopathic SGA/FGR, excluding cases explained by CPM may increase the power of association studies. When identified prenatally, CPM may signify that the pregnancy is at increased risk for complications depending on the extent of the abnormality and the chromosome(s) involved. For example, CPM of trisomy 8 has low risk of complications (59), while that of trisomy 16 is associated with a high risk for FGR and PE (10, 60–62). Additionally, there is an increased risk of UPD in the diploid cell population which can be associated with imprinting disorders; for example, upd(7)mat and upd(20)mat are associated with FGR and several long-term health complications (63, 64). Reassuringly, follow-up studies of cases of CPM without UPD suggest that most growth-restricted infants tend to have catch-up growth, normal neurodevelopment, and no global developmental delay (41, 65–67). Identifying cases that were growth-restricted due to CPM can inform further long-term outcome studies, particularly in relation to specific trisomies, to improve our understanding of the developmental trajectories and risks for complications in affected infants, and address the clinical utility of screening for CPM and UPD in cases of FGR.

Our findings also provide evidence that CNVs impacting genes relevant to growth or placental function may contribute to idiopathic SGA. In contrast to findings of aneuploidy CPM, the CNVs identified in our study were germline alterations and may therefore have clinical implications beyond birth. Future studies profiling CNVs associated with SGA or FGR may add to ours and improve the annotation of CNVs found in cases of obstetric complications, for which information is largely absent in population databases. Given the widespread use of non-invasive methods to detect placental DNA in maternal blood and the development of methods to identify CNVs from these samples (68–70), the feasibility of identifying pathogenic CNVs prenatally is increasing. This will have relevant implications for both predicting pregnancies at risk of FGR and its associated complications and for post-natal counselling if CNVs are not confined to the placenta. Additional research on the incidence and impact of CNVs on obstetric outcomes is thus needed to assess the potential clinical utility of this information.

Conclusions

Overall, we find consistent evidence that trisomy and segmental aneuploidy confined to the placenta are an important cause of poor fetal growth, and that rare germline CNVs overlapping genes of functional interest may also underlie a subset of idiopathic SGA cases. Together, these genomic imbalances may explain approximately 18% of SGA cases in our study population, and additional studies to evaluate the

clinical utility of screening for these abnormalities are warranted. Increased placental CNV load may not commonly impact fetal growth, however studies with larger sample sizes may help elucidate whether subgroups of SGA/FGR are linked to placental CNV load.

Abbreviations

CGH, comparative genomic hybridization; CNV, copy number variant; CPM, confined placental mosaicism; DMR, differentially methylated region; FDR, false-discovery rate; FGR, fetal growth restriction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MDS, multidimensional scaling; MLPA, multiplexed ligation-dependent probe amplification; PE, preeclapmsia; PlGF, Placental growth factor; SGA, small-for-gestational age; SNP, single nucleotide polymorphism; UPD, uniparental disomy; VUS, variant of uncertain significance

Declarations

Ethics approval and consent to participate

Ethics approval for use of human research subjects in this study was obtained from the University of British Columbia/Children's and Women's Health Centre of British Columbia Research Ethics board (H17-01545) and from the Hospital for Sick Children (1000038847) and Mount Sinai Hospital (05-0038-E) Research Ethics boards. Informed written consent was obtained from all study participants.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors report no conflict of interest.

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Author's contributions

GFDG contributed to the study design, prepared samples for microarray, performed microsatellite genotyping and data analysis, interpreted results, and wrote the manuscript draft. YY and EAB contributed to data analysis. JW performed initial data processing. SC prepared samples for microarray analysis and collected clinical information. RW provided samples and clinical information. PVD and HB contributed to subject recruitment. ERS contributed to study design, and interpretation of results. RKCY and WPR conceived and supported the study and contributed to the data analysis and interpretation of results. All authors read and provided critical revisions of the manuscript and approve the final version.

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Figures

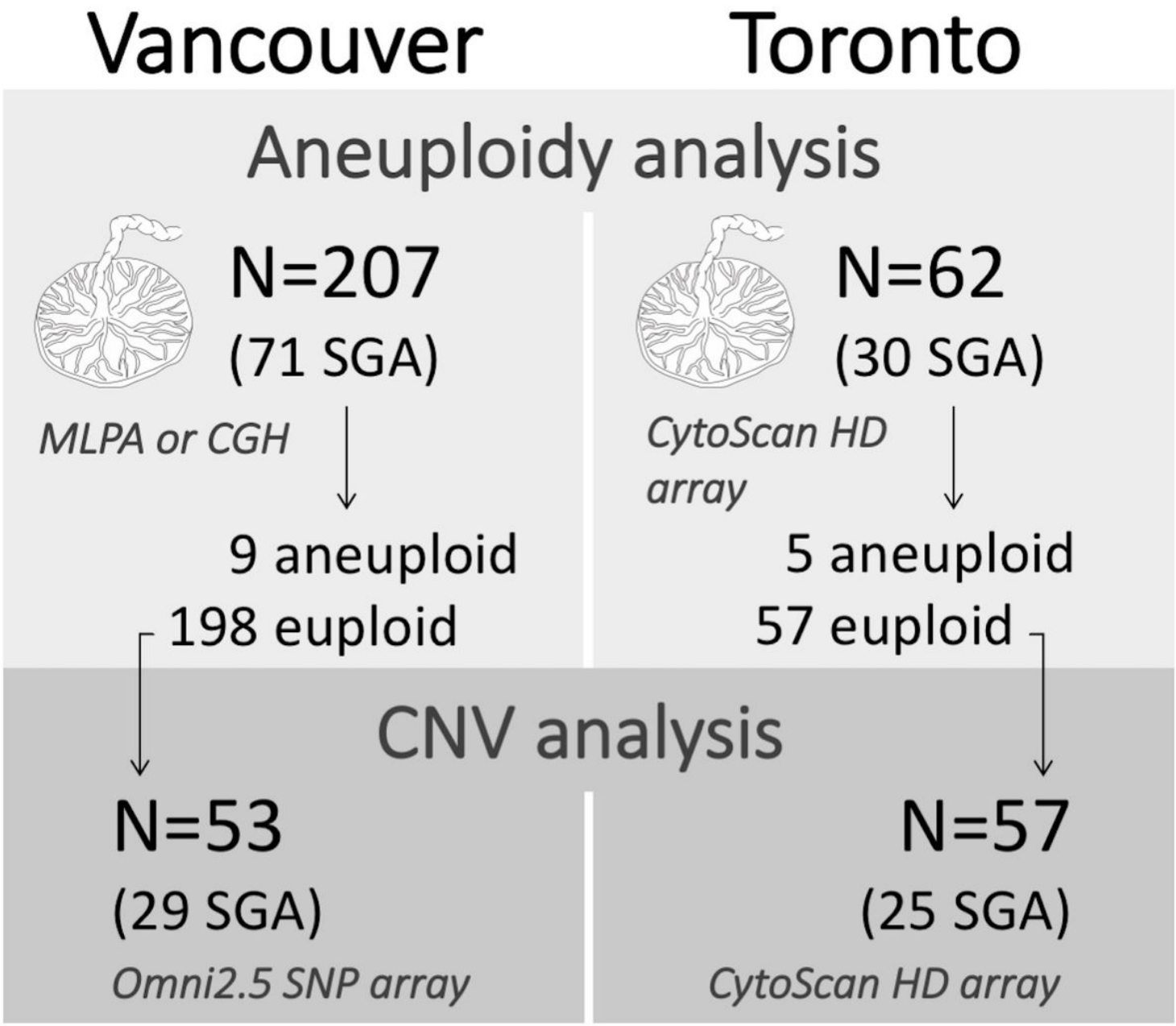

Figure 1

Schematic of the study design, including methods and sample sizes used in both cohorts in this study. Genetic assessment methods are italicized. CGH, comparative genomic hybridization; MLPA, multiplexed ligation-dependent probe amplification; QC: quality checks.

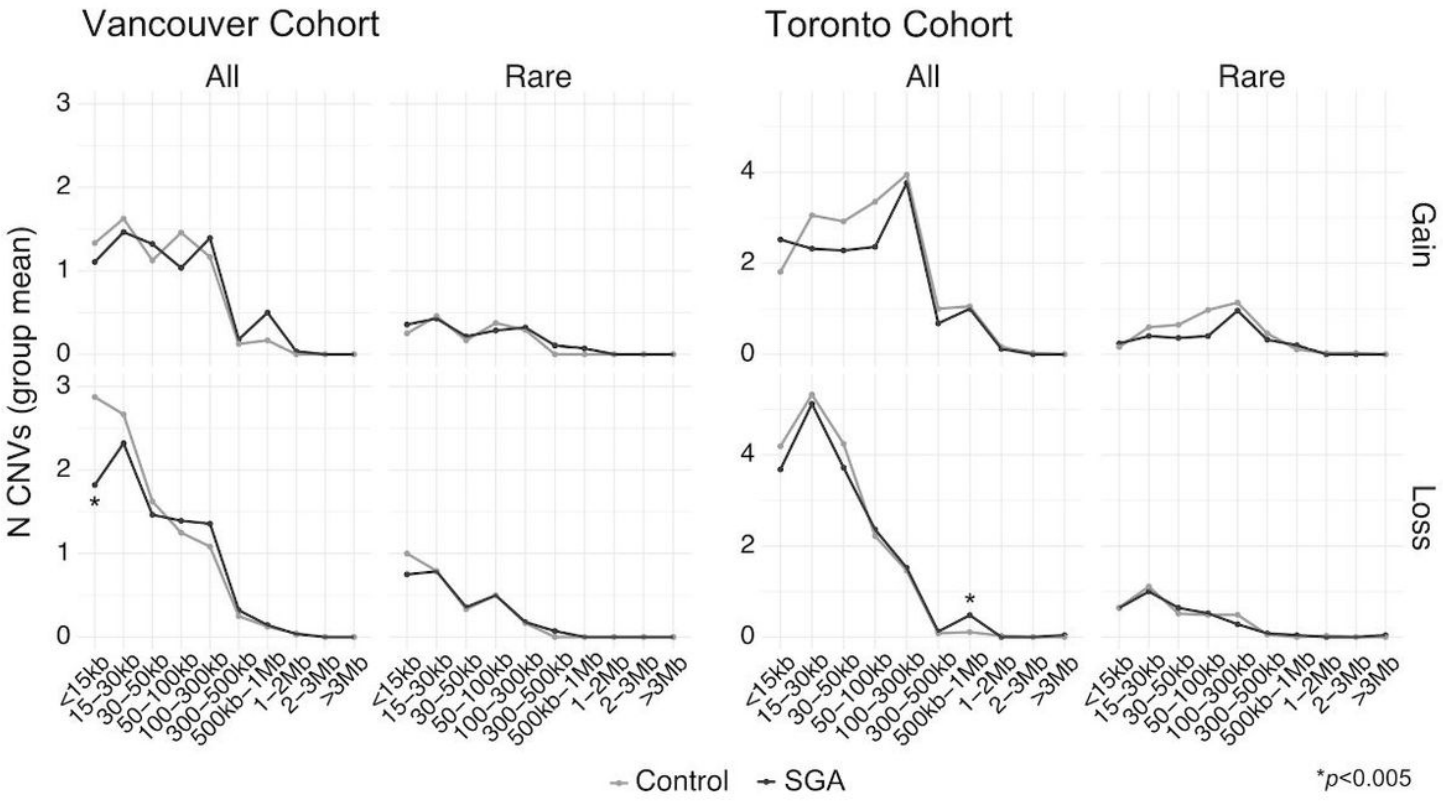

Figure 2

Sizes of placental CNVs from control and SGA pregnancies. Plots depict the mean number of CNVs per study group at different size bins in the Vancouver and Toronto cohorts, in all CNVs or exclusively rare CNVs, and separated by gains and losses. Overall, there is no consistent difference in the sizes of CNVs between SGA and control placentas. p-values calculated by Mann-Whitney U-test.

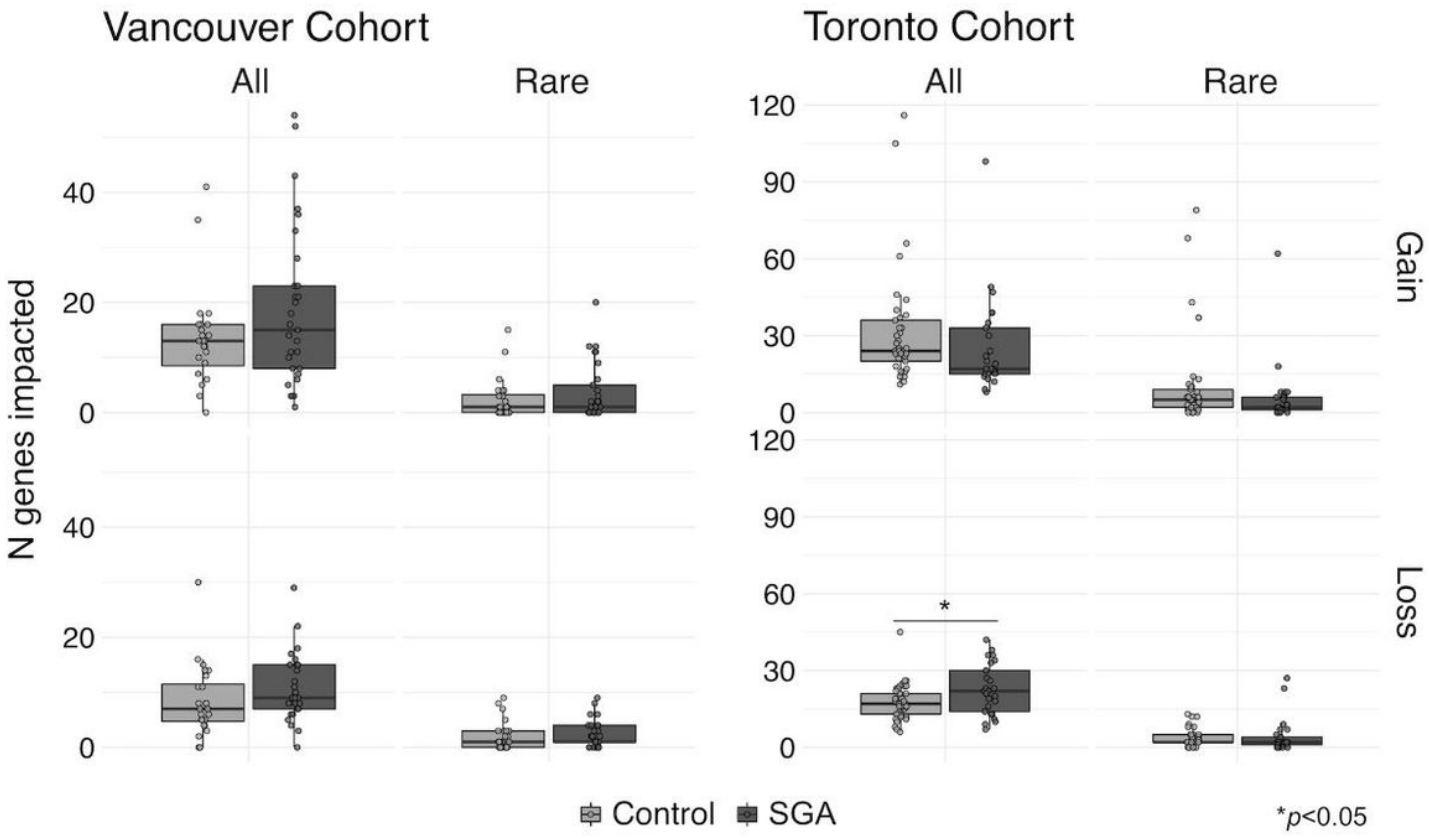

Figure 3

Total number of genes impacted by placental CNVs from control and SGA pregnancies. The cumulative total of unique RefSeq genes impacted by CNVs for each case in the Toronto and Vancouver cohorts are shown, separated by all CNVs or exclusively rare CNVs, and by gains and losses. Toronto cohort SGA placentas had slightly more genes affected by losses than controls. A similar trend was found in Vancouver cohort, but the difference was not statistically significant. p-values calculated by Mann-Whitney U-test.

Supplementary Files

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