OBSTETRICS

Characteristics and mode of inheritance of pathogenic copy number variants in prenatal diagnosis

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Matthew Hoi Kin Chau, MSc; Ye Cao, MD, PhD; Yvonne Ka Yin Kwok, PhD; Samantha Chan, BSc; Yiu Man Chan, MD; Huilin Wang, PhD; Zhenjun Yang, MEng; Hoi Kin Wong, MPhil; Tak Yeung Leung, MD; Kwong Wai Choy, PhD

BACKGROUND: Microdeletions and microduplications can occur in any pregnancy independent of maternal age. The spectrum and features of pathogenic copy number variants including the size, genomic distribution, and mode of inheritance are not well studied. These characteristics have important clinical implications regarding expanding noninvasive prenatal screening for microdeletions and microduplications.

OBJECTIVES: The aim was to investigate the spectrum and characteristics of pathogenic copy number variants in prenatal genetic diagnosis and to provide recommendations for expanding the scope of noninvasive prenatal screening for microdeletions and microduplications.

STUDY DESIGN: This was a retrospective study of 1510 pregnant women who underwent invasive prenatal diagnostic testing by chromosomal microarray analysis. Prenatal samples were retrieved by amniocentesis or chorionic villus sampling and sent to our prenatal genetic diagnosis laboratory for chromosomal microarray analysis. The risk of carrying a fetus with pathogenic copy number variants is stratified by the patients' primary indication for invasive testing. We searched the literature for published prenatal chromosomal microarray data to generate a large cohort of 23,865 fetuses. The characteristics and spectrum of pathogenic copy number variants including the type of aberrations (gains or losses), genomic loci, sizes, and the mode of inheritance were studied.

RESULTS: Overall, 375 of 23,865 fetuses (1.6%) carried pathogenic copy number variants for any indication for invasive testing, and 44 of them (11.7%) involve 2 or more pathogenic copy number variants. A total of 428 pathogenic copy number variants were detected in these fetuses, of which 280 were deletions and 148 were duplications. Three hundred sixty (84.1%) were less than 5 Mb in size and 68 (15.9%) were between 5 and 10 Mb. The incidence of carrying a pathogenic copy number variant in the high-risk group is 1 in 36 and the low-risk group is 1 in 125. Parental inheritance study results were available for 311 pathogenic copy number variants, 71 (22.8%) were maternally inherited, 36 (11.6%) were paternally inherited, and 204 (65.6%) occurred de novo.

CONCLUSION: Collectively, pathogenic copy number variants are common in pregnancies. High-risk pregnancies should be offered invasive testing with chromosomal microarray analysis for the most comprehensive investigation. Detection limits on size, parental inheritance, and genomic distribution should be carefully considered before implementing copy number variant screening in expanded noninvasive prenatal screening.

Key words: chromosomal microarray, microdeletion, microduplication, prenatal diagnosis

Prenatal testing has been revolutionized by 2 major advances: chromosomal microarray analysis (CMA) and noninvasive prenatal screening (NIPS). Chromosomal microarray analysis is used to investigate copy number variants (CNVs) at much higher resolution than standard cytogenetic analysis, and it is currently recommended for all women undergoing invasive testing.[1]

Many CNVs remain as benign variants within the population, while some are pathogenic copy number variants (pCNVs) and contribute to genomic disorders.[2] On the other hand, NIPS sequences the circulating cell-free DNA in the maternal plasma and is well established for screening of fetal chromosomal aneuploidies with high sensitivities and specificities. However, the performance of expanded NIPS for screening of CNVs is not well evaluated.

The clinical utility of NIPS may be improved by expanding the scope of screening to include specific microdeletions, which is technically feasible,[3] although more assessment of the accuracy and efficacy is warranted. Several recent studies have showed the possibility of using NIPS to detect fetal CNVs with higher sequencing depth, but this will increase the cost and reduce feasibility for clinical application.[4–6]

The current detection limit of standard NIPS for pCNV proposed by various studies may be larger than 5 Mb.[4,6,7] Moreover, there are 2 methods for NIPS; the genome-wide z-score counting method and the targeted single-nucleotide polymorphism (SNP)-based method, the latter of which can distinguish between a CNV that has arisen from a paternal or maternal chromosome.

Currently there is no consensus on the size cutoff for CNV detection in expanded NIPS, its required sequencing depth, and whether to perform detection of genome-wide pCNV or limiting it to a targeted approach. These questions can be addressed by studying the spectrum and characteristics of pCNVs in prenatal diagnosis because they primarily depend on size, distribution across the genome, and their mode of inheritance.

In this study, we examine the frequency and spectrum of pCNVs across most common clinical indications for invasive prenatal testing by CMA and study the physical characteristics and mode of inheritance of the pCNVs detected among women undergoing invasive prenatal diagnosis. Our findings

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AJOG at a Glance

Why was the study conducted?

- For any presenting clinical indication for prenatal testing, should pregnant women choose noninvasive prenatal screening (NIPS) or invasive diagnosis?
- Expanding of the scope of NIPS for the detection of microdeletion and microduplication syndromes in fetuses is controversial, and there is no consensus on resolutions of NIPS and which syndromes to be included.

Key findings

- We study the incidence, spectrum, and characteristics including genomic loci, size, and mode of inheritance to provide insight to test selection and NIPS-expanded screening.

What does this add to what is known?

- The size characteristics, proportion of inheritance, and genomic distribution of the detected pathogenic copy number variants have implications for next-generation sequencing-based diagnostic tests and noninvasive prenatal screening tests.

have implications in genetic counseling, test selection, and the development of NIPS-expanded CNV screening and other next generation sequencing (NGS)-based screening or diagnostic tests.

Materials and Methods

Subjects

Pregnant women referred for prenatal molecular cytogenetic investigation for various clinical indications, including advanced maternal age (AMA; age ≥35 years), abnormal fetal ultrasound findings (AUS), high-risk fetal aneuploidy screening results (DSS), maternal anxiety (MA), a family history or previous child with chromosomal abnormalities (FHX), high risk by NIPS (NIPS-HR) on either chromosomal or subchromosomal level, and other rare or unspecified indications.

All patients have given written informed consent for storage and genetic analysis of chorionic villus samples and amniotic fluid samples. In fetuses with multiple indications, the primary indication was classified by the indication with the higher a priori risk for pCNVs or more severe indication for prenatal testing (AUS > NIPS-HR > DSS > FHX > AMA > MA > others). We examine the incidence of chromosomal abnormalities and pCNVs across different indications for invasive testing.

A total of 1517 prenatal samples were received by the Prenatal Genetic Diagnosis Laboratory, The Chinese University of Hong Kong, between January 2012 and December 2017 for invasive prenatal molecular genetic investigation. Seven cases were excluded from the analysis because of the following reasons: 2 replicates, 4 canceled, and 1 failed test. Chromosomal microarray results were available in 1510 fetuses and performed on 502 chorionic villi (33.2%) and 1008 (66.8%) amniotic fluid samples.

DNA was extracted using manufacturer protocols (DNeasy blood and tissue kit; Qiagen, Dusseldorf, Germany). Quantitative fluorescence polymerase chain reaction was performed on all fetal samples to detect common aneuploidies (chromosomes 13, 18, 21, X, Y), polyploidy, and maternal cell contamination. In cases with copy number variant findings, CMA was also performed on parental samples to determine the mode of inheritance of the aberrations.

Customized targeted high-resolution 8 × 60 K (comparative genomic hybridization) or 8 × 60 K (comparative genomic hybridization plus SNP) oligonucleotide arrays (Fetal Chip version 1.2 or version 2.0; Agilent Technologies Inc, Santa Clara, CA) were used for the CMA analysis. The fetal chips were designed with the intention of targeting more than 100 known microdeletion and microduplication syndromes while minimizing the detection of common nonpathogenic variants and providing backbone coverage of the genome at a resolution of 100 kb (http://www.obg.cuhk.edu.hk/wp-content/uploads/Fetal-DNA-Chip-v2.0_Leaflet.pdf).

Data interpretation

There is no consensus for a cutoff for the detection of pCNVs in NIPS because the resolution depends on the sequencing depth of NIPS. Because we aimed to examine the full spectrum of pCNVs to provide insight on this matter, we examined pCNVs <5 Mb and those between 5 and 10 Mb for a wider range of aberrations. Because we are examining the characteristics of the pCNVs for implications in expanded NIPS, we excluded mosaic CNVs from the analysis because they are currently outside the scope of NIPS.

The clinical significance of the detected CNVs were classified in accordance with the American College of Medical Genetics and Genomics standards and guidelines.[8,9] The CNVs were compared with known CNVs from publicly available databases including Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home), DECIPHER (https://decipher.sanger.ac.uk/), OMIM (http://www.omim.org), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), published cases, and the laboratory internal database.[10]

Well-known CNVs encompassing susceptibility loci known to result in variable expressivity and incomplete penetrance were also classified as pathogenic CNVs. Although the full clinical manifestation may not be precisely predicted, the pathogenic nature of the CNV is not in question.[8]

Published data summary

We performed a literature search focused on prenatal diagnostic testing using CMA. Pubmed (2006−2018) and EMBASE (2006−2018) databases were

TABLE 1
Frequency of clinical indications and pathogenic copy number variants (<10 Mb) detected by chromosomal microarray analysis in 1510 prenatal referrals at our center, 22,355 cases from the literature, and total 23,865 cases

Indication for prenatal diagnosis	Our study, n	Cases with pCNV <10 Mb, n (%) [95% CI]	Literature, n	Cases with pCNV <10 Mb, n (%) [95% CI]	Total, n	Cases with pCNV <10 Mb, n (%) [95% CI]
High-risk indications	1365	61 (4.5) [3.5–5.7]	7931	197 (2.5) [2.2–2.9]	9296	258 (2.8) [2.5–3.1][a]
Abnormal ultrasound findings	990	47 (4.8) [3.6–6.3]	3709	144 (3.9) [3.3–4.6]	4699	191 (4.1) [3.5–4.7]
High-risk aneuploidy screening	152	3 (2.0) [0.7–5.6]	3154	33 (1.0) [0.7–1.4]	3306	36 (1.1) [0.8–1.5]
Family history[b]	126	4 (3.2) [1.2–7.9]	1068	20 (1.9) [1.2–2.9]	1194	24 (2) [1.4–3]
NIPS high risk	97	7 (7.2) [3.5–14.2]	0	0	97	7 (7.2) [3.5–14.2]
Low-risk indications	145	0 (0) [0–2.6]	14,424	117 (0.8) [0.7–1.0]	14,569	117 (0.8) [0.7–1.0][a]
Advanced maternal age (≥ 35 y)	27	0 (0) [0–12.5]	9965	79 (7.9) [6.3–9.8]	9992	79 (0.8) [0.6–1]
Maternal anxiety	21	0 (0) [0–15.5]	3283	18 (0.6) [0.4–0.9]	3304	18 (0.5) [0.3–0.9]
Others[c]	97	0 (0) [0–3.8]	1176	20 (1.7) [1.1–2.6]	1273	20 (1.6) [1–2.4]
Total	1510	61 (4.0) [3.2–5.2]	22,355	314 (1.4) [1.3–1.6]	23,865	375 (1.6) [1.4–1.7]

CI, confidence interval; *NIPS*, noninvasive prenatal screening; *pCNV*, pathogenic copy number variant.

[a] Pearson's χ^2 test, $P < .001$; [b] Family history: genetic conditions in the parents, previous child with chromosomal abnormalities, abnormal fetal karyotype; [c] Others: rare and unspecified indications, isolated ultrasound soft markers: choroid plexus cysts, echogenic foci in heart or bowel, short long bones, absent or hypoplasia of nasal bone, single umbilical artery, mild ventriculomegaly, mild pyelectasis, increased nuchal fold, enlarged cisterna magna.

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searched electronically. The search captured citations using MeSH keywords and word variants of prenatal, microarray, and array comparative genomic hybridization.

The eligibility criteria for study selection was CMA used during prenatal diagnosis and the clinical indications and CMA results are available. CMA is performed on any indication and not limited to cases referred by abnormal ultrasound findings or any specific structural defect. Our approach is to capture a combined cohort to represent an obstetric population who undergo invasive prenatal testing.

Studies with patient referrals from at least 3 types of indication for invasive testing are included and studies focusing on 1 specific indication (eg, ultrasound structural abnormalities only) were not selected. A total of 22,355 fetuses from 14 selected studies were combined with our local data (selected studies and cases are listed in Appendix Table 1)[11–24] for the analysis of the incidence and the characteristics of the spectrum of pCNVs.

Fetuses with increased nuchal translucency (≥3.5 mm) are also included in the AUS group because of the existing classification by various studies[11,14,15]. Considering the differences in classification of CNVs by various studies, clinically significant findings are also termed as pCNV for the purpose of the combined study. To the best of our knowledge, no clinically significant CNVs classification in the literature was downgraded to benign or variants of uncertain significance during the preparation of this manuscript.

Statistical analyses

The incidence of pCNVs referred by each clinical indication for invasive testing is shown as proportions with 95% confidence intervals calculated with the Wilson score method without continuity correction.[25] The pCNVs were further stratified and described including the type of aberration, the size, the genomic loci, and the mode of inheritance. All statistical analyses were performed with the statistical software package SPSS 25.0 (IBM SPSS Statistics for Windows, version 25.0; IBM Corp, Armonk, NY).

Results

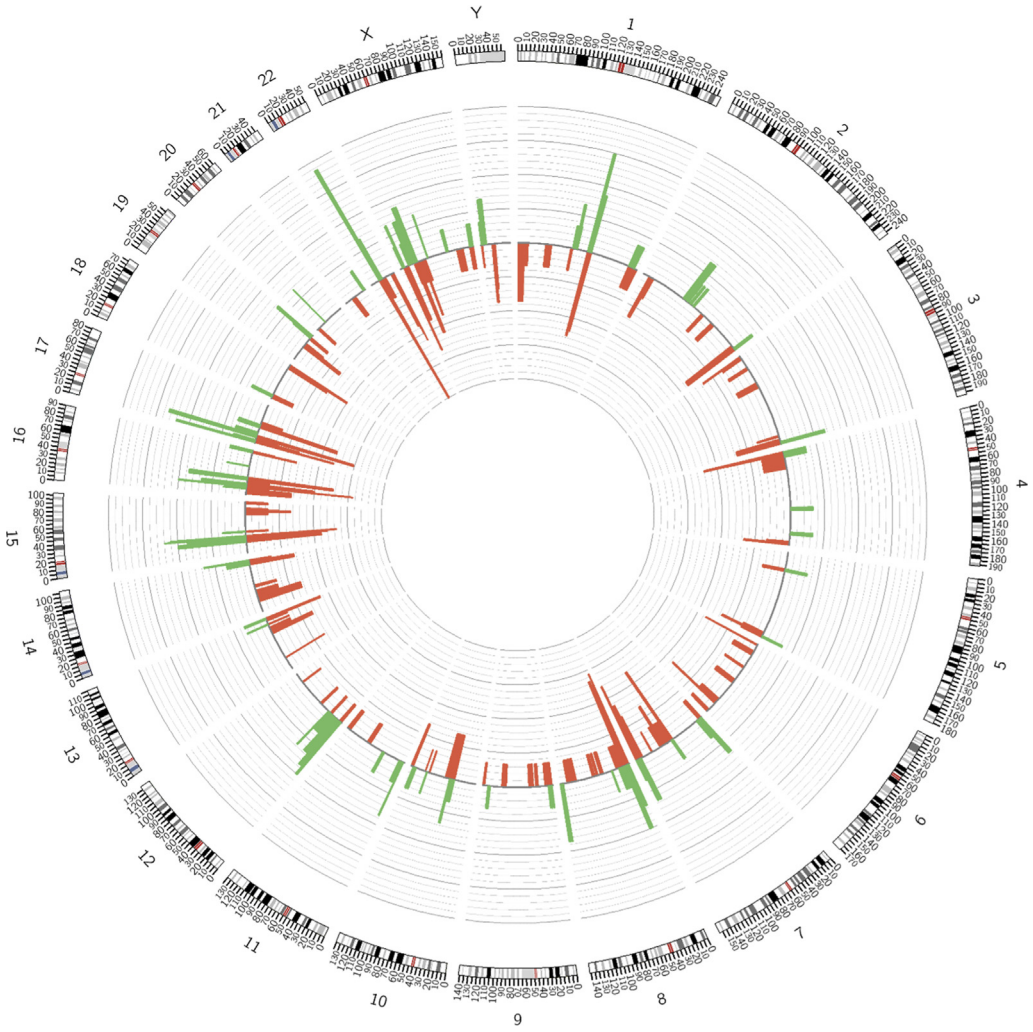
Chromosomal abnormalities and pathogenic CNVs

In our center, among 1510 cases undergoing invasive prenatal diagnosis by CMA, common aneuploidies were detected in 38 cases (trisomy 13, trisomy 18, trisomy 21), sex chromosomal aneuploidies in 23 cases, structural imbalance (≥10 Mb) in 19 cases, and mosaic aberrations in 28 cases (Appendix Table 2). In 61 fetuses, pCNVs (<10 Mb) were detected.

The incidence of fetuses carrying pCNVs (<10 Mb) is 61 of 1365 (4.5%) in the high-risk group (AUS, DSS, NIPS-HR, FHX) and 0 of 145 (0%) in the low-risk group (AMA, MA, others/unspecified) (Table 1). Four of these pCNVs (6.6%) were detected as mosaic in our center. Variants of uncertain significance were detected in 66 fetuses (4.4%).

In the combined data, pCNVs (<10 Mb) were detected in 375 of 23,865 fetuses (1.6%). The incidence in the high-risk group (AUS, DSS, FHX, NIPS-HR) is 258 of 9296 (2.8%, 1 in 36) and is significantly higher than 117 of 14,569 (0.8%, 1 in 125) in the low-risk group (AMA, MA, others/unspecified) ($P < .001$). The incidence of pCNV findings of the combined data stratified by primary indications are shown in Table 1. The incidence of pCNVs in fetuses referred with abnormal ultrasound findings is 4.1% (191 of 4699) and those not indicated by ultrasound

FIGURE
Distribution of pathogenic copy number variants across the genome



The distribution of pathogenic copy number variants across the genome detected in 23,865 cases. In total, 419 nonmosaic and 9 mosaic copy number variants were detected and classified as pathogenic. *Green bars* represent copy number gains and *red bars* represent copy number losses encompassing the chromosomal bands. The height represents the frequency of the pathogenic copy number variations in log2 scale.

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structural abnormalities is 1.0% (184 of 19,166). Pregnancies referred by NIPS—high risk results, abnormal ultrasound findings, and a family history had the highest incidence of carrying a fetus with a pCNV. The incidence of pCNVs was not significantly different between those referred by AMA and those referred by MA.

Genomic distribution of pathogenic CNVs

Overall, 428 pCNVs (<10 Mb) were detected in 375 of 23,865 fetuses: 280 deletions and 148 duplications. Forty-four fetuses (11.7%) had multiple (2 or more) pCNVs detected by CMA. Only 9 pCNVs were reported to be mosaic, and they are excluded from the subsequent analysis on size and inheritance because they are outside the scope for consideration of the expanded NIPS.

The frequency and distribution of aberrations across each chromosome are shown in the Figure. Both risk groups follow the same trend of distribution across the genome, with highest frequencies on chromosome 22. The chromosome bands with the most aberrations detected were 22q11.21 deletions (47 cases), 22q11.21 duplications (24 cases), 16p13.11 deletions (13 cases), 17p12 deletions (11 cases), 1q21.1 duplication (11 cases), and 8p23.3 deletions (10 cases). Some of these hotspots were contributed by pCNVs encompassing recurrent susceptibility loci such as 22q11.2 duplications (online inheritance in man [OMIM] number 608363), 1q21.1 duplications (OMIM number 612475) and 16p13.11 deletions.[26] Others are caused by known recurrent

microdeletion syndromes such as 22q11.21 deletions (OMIM number 188400), 17p12 deletions (OMIM number 162500), and 8p23.3 inverted duplication deletions.[27]

Size of pathogenic CNVs

The size distribution of the pCNVs is described in Table 2. Most pCNVs (352 of 419 [84.0%]) are less than 5 Mb in size and 67 of 419 (16.0%) were between 5 and 10 Mb. The mean sizes of the gains and losses in the group <5 Mb are 1.5 ± 1.2 Mb (mean size ± SD) and 1.8 ± 1.3 Mb (mean size ± SD), respectively. The mean sizes of the gains and losses in the group 5–10 Mb are 7.2 ± 1.2 Mb and 7.1 ± 1.4 Mb.

Inheritance pattern of pathogenic CNVs

The inheritance pattern of the gains and losses are listed in Table 3. Parental inheritance information was available in 311 of 419 pCNVs. In the 106 gains with inheritance information, 52 gains (49.1%) were de novo, 32 (30.2%) were maternally inherited, and 22 (20.8%) were paternally inherited. In the 205 losses with inheritance information, 152 (74.1%) were de novo, 39 (19.0%) were maternally inherited, and 14 (6.8%) were paternally inherited. Moreover, of the 71 pCNVs of maternal origin, the chromosomes with the highest frequency with aberrations were chromosome X (20/71), chromosome 17 (12/71), and chromosome 22 (9/71).

TABLE 2
The characteristics of the 419 nonmosaic pathogenic copy number variants (<10 Mb) detected in 23,865 fetuses

Variable	Count		Mean size, kb		Median size, kb		SD, kb	
Size of pCNVs, Mb	Gain	Loss	Gain	Loss	Gain	Loss	Gain	Loss
<5	126	226	1499	1834	1246	1603	1201	1280
5–10	17	50	7179	7096	7100	6606	1151	1411

pCNV, pathogenic copy number variant.
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Comment

Principal findings

This is the largest cohort showing that the overall incidence of pCNV is 1.6% among women undergoing prenatal diagnosis, and it is enriched in the high-risk group. The majority of the pCNVs are less than 5 Mb in size, which are beyond the detection resolution limit of current standard NIPS. The genomic distribution of the pCNVs show recurrent hotspot deletions and duplications. There is a significant portion of pCNVs (34.4%) inherited from the parents.

Clinical implications

pCNVs are common in pregnancies, and the incidence is increased in high-risk indications. The majority of pCNVs detected were smaller and beyond the detection limit of various NIPS platforms currently available. Thereby, standard NIPS is not suitable for high-risk pregnancies and invasive testing should be offered. Generally, NIPS is performed early in gestation, before structural abnormalities can be detected by ultrasonography. Even in pregnancies not indicated by abnormal ultrasound findings, they may still have 1.0% chance of having a fetus carrying a pathogenic CNV as shown in our data. This risk should be discussed during genetic counseling for patients to understand the detection limitations of the current noninvasive prenatal screening tests.

Based on our findings, the majority of pCNVs were less than 5 Mb in size and not involving common trisomies; thus, standard NIPS cannot reliably detect these abnormalities. The performance of pCNV detection by NIPS is only beginning to be validated for accuracy and clinical purposes.[6,7,28,29]

Studies evaluating the performance of standard NIPS in the detection of prenatal pCNVs showed that most of the pCNVs >6 Mb in size (83%) can be detected by standard NIPS, but only 20% of the CNVs <6 Mb could be detected.[4] Similarly, Li et al[7] detected 90.9% of CNVs >5 Mb in CMA-positive fetuses but only 14.3% of those with CNVs <5 Mb.

The positive predictive values are low and false-positive rates are high. Increase in false-positive rates would also increase the number of potentially unnecessary invasive diagnostic tests and will result in a false-positive result and maternal anxiety.[30] The sensitivity and positive predictive value for nonrecurrent and small CNVs are poor, which may result in a false sense of reassurance that no pCNV is present.[31]

A considerable proportion of the pCNVs were inherited from the parents. CNVs of maternal origin complicates NIPS because the cell-free DNA of placental origin will be diluted in an excess of maternal DNA, which contains the same deletion or duplication. The presence of a maternal CNV can also lead to false-positive NIPS results, regardless whether the fetus inherited the CNV.[32]

We could not obtain phenotype information from parental clinical evaluations in cases in which the fetus has inherited a pCNV from one of the parents. Contrary to the Z-score counting method, SNP-based NIPS has the advantage of being able to distinguish between a CNV that is of maternal origin or placental origin. However, in parents with any clinical phenotype suggestive of a genetic etiology, the mainstay should be invasive prenatal testing with parental confirmation.

The distribution of pathogenic CNVs is not random throughout the genome, although they can occur on every chromosome. Pathogenic CNVs occurred most frequently on chromosome 22q11.2, 17p12, 16q13.11, and 1q21.1 and are mostly contributed by low copy repeat regions.[33] There was an abundance of recurrent pCNVs in susceptibility loci including deletions and duplications of 1q21.1, 16p11.2, duplications of 22q11.2, and deletions of 16p13.11.

The incomplete penetrance of susceptibility loci certainly invokes

TABLE 3
Mode of inheritance for 419 nonmosaic pathogenic copy number variants (<10 Mb) detected in 23,865 fetuses

	Inheritance of gains[a]				Inheritance of losses[b]			
	De novo	Mat	Pat	NA	De novo	Mat	Pat	NA
Total	52	32	22	37	152	39	14	71

Mat, maternal; *NA*, not available; *Pat*, paternal.

[a] One-sample χ^2 test: gains: $\chi^2(2) = 13.2$, $P = .001$; [b] One-sample χ^2 test: losses: $\chi^2(2) = 158.2$, $p < .001$.

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challenges in genetic counseling and is complicated even further when the CNVs are inherited.[26,34] The distribution of pCNVs followed a similar trend in both the high-risk and low-risk groups. We observed that 22q11.2 region is the locus with the highest frequency of being involving in a copy number variation.

Studies have shown that targeted SNP-based NIPS is superior in the detection of microdeletions in the 22q11.2 region with sensitivity greater than 90%.[28,35] However, the positive predictive value was estimated to be only 5.3−19.6%.[28,35] The positive predictive value can be improved to 44.2% with increased sequencing depth.[36] Nonetheless, all the NIPS studies demonstrate NIPS both by counting or SNP approach cannot accurately detect pathogenic CNVs <5 Mb without increasing sequencing depth.

Our data support the initial approach of expanding the scope of NIPS by focusing on the genomic loci with recurrent well-known microdeletions and microduplications and consider the remaining genome-wide pCNVs only when technically feasible.[3]

Screening of clinically significant CNVs would increase the clinical utility of NIPS. While specific microdeletions and microduplications are rare, pCNVs are collectively more common than whole chromosomal aneuploidies and can have equal or more severe phenotypes.[37] A study of women's preference for NIPS vs CMA with intermediate or high risk by Down syndrome screening and not indicated by structural anomalies, more than half (62.2%) would choose NIPS over CMA for a simple aneuploidy assessment over a comprehensive diagnostic test.[38]

The main advantage of NIPS is that invasive sampling is not required, and the risk of sampling procedure-related miscarriage is avoided. However, this advantage is becoming less of a factor because the current risk of procedure-related miscarriage is much lower at 0.11% and 0.22% for amniocentesis and chorionic villi sampling and are not significantly different from those who do not undergo any invasive procedure.[39] Furthermore, any positive NIPS results will still require an invasive prenatal test to confirm the finding because of the screening nature of NIPS.

A recent randomized clinical trial studied the miscarriage rates in women who performed invasive testing after a positive NIPS result for trisomy 21 vs immediate invasive testing in those with pregnancies at high risk of trisomy 21 as identified by first-trimester combined screening. The miscarriage rates between these 2 groups showed no significant difference, further showing evidence that safety is no longer an argument for NIPS over direct invasive testing.[40] Women at high risk pursuing invasive testing directly can save time and test cost because the average reporting time of NIPS tests is 3−10 working days.

The widespread use of NIPS will likely increase the positive attitudes of pregnant women to opt toward NIPS.[41,42] A decrease in invasive diagnostic procedures in the post-NIPS era might lead to an increase in missed diagnoses of potentially NIPS undetectable pCNVs or genetic aberrations, which may have significant phenotypical consequences.[43] We show recurrent pCNVs hotspots and the size of the pCNVs are too small to be reliably detected by current NIPS methodologies. Genome-wide CNV screening with NIPS at present is not ready for clinical implementation.

Patients are often given the dilemma of having to choose between the NIPS and CMA. It is difficult to compare a screening test with an invasive diagnostic test directly. However, considering the scope of detection of genetic abnormalities by invasive sampling with CMA and the reduction of procedure-related miscarriage by NIPS, indeed the advantages of CMA outweigh that of NIPS. CMA should be offered to all women undergoing invasive diagnostic procedures.[40]

Moreover, our findings also have implications on NGS-based whole genome sequencing tests on invasive samples, which may soon replace CMA and karyotyping because they have the potential to detect not only genome-wide chromosomal abnormalities and CNVs but also balanced structural rearrangements in a single test.[44−46]

Strengths and limitations

A major strength is that this study has the largest combined cohort including those from a variety of different clinical indications for invasive testing to represent the obstetric population undergoing invasive prenatal diagnosis. The CMA findings represent a spectrum of pCNVs identified among prenatal testing. This is the first study to describe the proportion of inherited vs de novo pCNVs.

Limitations include the types and versions of CMA used in the various selected studies are heterogeneous. The microarray platforms used by the various studies are shown in Appendix Table 3; their backbone resolutions vary from 100 kb to 1 Mb. The differences in targeted probe content, density, and coverage of the microarrays may affect the size and CNVs detected.[47] A portion of cases (38.4%) had CMA performed with genome-wide resolution at 1 Mb resolution, so there is a possibility that we underestimated the incidence of pCNVs. We were not able to further stratify abnormal ultrasound findings by specific structures or organs and its risk

of carrying a fetus affected with a pCNV because detailed phenotype were not available.

Conclusions

Clinically significant microdeletions and microduplications occur in 1.6% of women undergoing invasive prenatal diagnosis, regardless of maternal age; therefore, screening of clinically relevant microdeletions would be beneficial for the general obstetric population.[41,45,48] Pathogenic CNVs constitute a group of diagnosable genetic abnormalities, and the cumulative risk is higher than Down syndrome, which are currently not routinely screened for. Our data highlights the size characteristics of the pCNVs are smaller than the detection limit of NIPS, and the proportion of maternal CNVs remains a challenge for false-positive or discordant NIPS results. Expanded NIPS tests with selected microdeletions or even genome-wide detection of pCNVs are available on the market, but the detection limits remain a concern. Future research should assess the clinical validity of expanded NIPS screening for a selection of common and well-known microdeletion and microduplication syndrome, focusing on the detection resolution and parental inheritance differentiation. Clinical implementation of CNV screening in expanded NIPS should be carefully considered. Accurate screening of pCNVs with NIPS would be beneficial in clarifying underlying genetic abnormalities in the developing fetus, better assess the risk for microdeletion and microduplication syndromes, and help prospective parents to make informed choices in further management and planning. ■

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Author and article information

From the Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong, China (Drs Cao, Kwok, Chan, Wang, Leung, and Choy, Mr Chau, Mr Yang, and Mr Wong); the Warwick Medical School at the University of Warwick, Coventry, United Kingdom (Ms Chan); the Department of Central Laboratory, Bao'an Maternity and Child Healthcare Hospital, Jinan University School of Medicine, Key Laboratory of Birth Defects Research, Birth Defects Prevention Research, and Transformation Team, Shenzhen, China (Dr Wang); the Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, China (Drs Wang and Choy and Mr Yang); and The Chinese University of Hong Kong-Baylor College of Medicine Joint Center for Medical Genetics, Hong Kong, China (Dr Leung and Choy).

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Corresponding author: Kwong Wai Choy, PhD. richardchoy@cuhk.edu.hk

APPENDIX TABLE 1

The detection of pathogenic copy number variants (<10 Mb) by chromosomal microarray analysis for different clinical indications in the current and previous published studies

	High-risk group								Low-risk group							
	Abnormal ultrasound, including high NT (≥3.5 mm) and anomalies		First- or second-trimester high risk for fetal aneuploidy screening		Family history		Noninvasive prenatal screening, high risk		Advanced maternal age		Maternal anxiety		Others/ unspecified		Total	
Study	Enrolled	pCNV cases	Enrolled	pCNV cases	Enrolled	pCNV cases	Enrolled	pCNV cases	Enrolled	pCNV cases	Enrolled	pCNV cases	Enrolled	pCNV cases	Enrolled	pCNV cases
Wapner et al[11]	755	45	729	12					1966	32			372	5	3822	94
Fiorentino et al[19]	95	5	29	0	50	1			1118	6	1675	11	33	0	3000	23
Armengol et al[15]	173	8	235	1	145	5			273	3	60	1	20	0	906	18
Oneda et al[18]	144	9	86	0	36	1			187	2	10	0			463	12
Lee et al[14]	194	15	26	0	51	5			1911	10	989	5			3171	35
Kan et al[12]	77	8	116	2							27	0			220	10
Maya et al[17]	102	2	2	0	58	1			61	0	46	1			269	4
Papoulidis et al[13]	685	8	258	2	100	1			592	4	128	0			1763	15
Van Opstal et al[16]			530	2					624	1			176	2	1330	5
Wu et al[20]	143	10			55	1			6	0			13	0	217	11
Sotiriadis et al[24]	648	19	334	5	216	4			719	6	310	0	552	13	2779	47
Konialis et al[23]	443	7	328	2	244				2107	12					3122	21
Scott et al[22]	62	3	478	7	77	0			393	3	29	0	10	0	1049	13
Coppinger et al[21]	188	5	3	0	36	1			8	0	9	0			244	6
Current study	990	47	152	3	126	4	97	7	27	0	21	0	97	0	1510	61
Total	4699	191	3306	36	1194	24	97	7	9992	79	3304	18	1273	20	23,865	375

To compare the clinically significant CNVs with our study, we categorized the inidications according to the definintions we describe in *Materials and Methods*. Values might differ from those of original publication because the reporting criteria include pathogenic or clinically relevant CNVs of <10Mb in size. *pCNV*, pathogenic copy number variant.

Chau et al. Pathogenic copy number variants in prenatal diagnosis. Am J Obstet Gynecol 2019.

APPENDIX TABLE 2
Detection of clinically significant abnormalities in prenatal referrals at our center

Abnormality	Cases
Common aneuploidies (+13, +18, +21)	38
Other rare aneuploidies	2
Mosaic aneuploidies	11
Aneuploidies involving 2 chromosomes	1
Sex chromosome aneuploidies	23
Mosaic sex chromosome aneuploidies	5
Structural imbalance (>10 Mb)	19
Mosaic structural imbalance (≥10 Mb)	7
Pathogenic CNV (<10 Mb)	43
Mosaic pathogenic CNV (<10 Mb)	3
Pathogenic CNV (<10 Mb) and structural imbalance (≥10 Mb)	13
Pathogenic CNV (<10 Mb) and mosaic pathogenic CNV (<10 Mb)	1
Pathogenic CNV (<10 Mb) and mosaic structural imbalance (≥10 Mb)	1
Variants of uncertain significance	66
Normal	1277
Total	1510

CNV, copy number variant.

Chau et al. Pathogenic copy number variants in prenatal diagnosis. Am J Obstet Gynecol 2019.

APPENDIX TABLE 3
The array platform utilized by our current study and of the selected studies

Study	Array platform	Array reporting criteria/resolution
Wapner et al[28]	44k custom oligonucleotide array Agilent or Affymetrix genome-wide human SNP array 6.0	(1) Overlap with disease-causing region, regardless of size (2) A copy-number variant of 1 Mb or greater in size in the pericentromeric or subtelomeric regions or in the genomic backbone, or a copy-number variant of less than 1 Mb in size in a nontargeted region but including a gene or portion of a gene implicated in a known chromosomal syndrome, an autosomal dominant Mendelian disorder, or an X-linked disorder.
Fiorentino et al[19]	CytoChip focus constitutional, blue gnome	Clinically significant CNVs. 100kb for disease causing region, 0.5Mb for genomic backbone coverage
Armengol et al[15]	Custom targeted BAC microarray	Pathologic & clinically relevant CNVs; 350 kb targeted disease-causing region
Oneda et al[18]	Affymetrix cytogenetics whole genome 2.7M array or Cytoscan HD array	Clinically significant CNVs; filter cutoff 20—100 kb
Lee et al[14]	Combimatrix molecular diagnostics CA3000 microarray slides, BAC aCGH, then confirmed by 105K oligonucleotide array or 60 k oligonucleotide array	Pathological imbalances; 1 Mb resolution (BAC) or 0.5 Mb (oligo), then confirmey 105 k oligo array
Kan et al[12]	NimbleGen CGX-135k	Clinically significant CNVs; 40 kb in disease-causing regions, 140 kb across genome
Maya et al[17]	Oligo 105 k signature (7% cases)/BAC4685 signature (87.7% cases)/unknown (5.3% cases)	Clinically significant causal CNVs; unspecified resolution
Papoulidis et al[13]	Cytochip focus constitutional, Illumina	Pathogenic/unknown penetrance. 100 kb in disease-causing region, 1 Mb across genome
Van Opstal et al[16]	300k Illumina HumanCyto SNP-12 (HCS) array	Causative findings and unexpected diagnoses; 300 kb resolution array; analyzed at a 0.5 Mb resolution
Wu et al[20]	Agilent 8X60K SurePrint G3 human CGH microarray/ Agilent 4X180K SurePrint prenatal research array	Pathogenic CNVs; 400 kb
Coppinger et al[21]	SignatureChip whole genome (BAC)/Signature PrenatalChip (BAC)	Clinically significant CNVs.
Sotiriadis et al[24]	BAC array/ oligonucleotide (oligo) array SurePrint G3 ISCA V2 CGH KIT, 8x60k (Agilent Technologies)	Pathogenic/likely pathogenic CNVs. 1114/2617 BAC array 1 Mb resolution, 1665/2617 oligonucleotide (oligo) array SurePrint G3 ISCA V2 CGH
Konialis et al[23]	CytoChip focus constitutional, BlueGnome, Cambridge, UK)	100 kb resolution in targeted disease-causing regions, 1 Mb backbone
Scott et al[22]	Agilent ISCA 8X60k, Agilent ISCA 8X60k (SUFWprenatal array)	Pathogenic CNVs and including reduced penetrance; genome coverage of 80 kb
Current study	Customized targeted high-resolution 4x44K (CGH) or 8x60K (CGH+SNP) oligonucleotide arrays (Fetal Chip v1.2 or v2.0; Agilent Technologies Inc, Santa Clara, CA)	Pathogenic CNVs; 100 kb genome wide coverage

aCGH, array comparative genomic hybridization; *BAC*, bacterial artificial chromosomes; *CNV*, copy number variant.

Chau et al. Pathogenic copy number variants in prenatal diagnosis. Am J Obstet Gynecol 2019.