

Research Highlight:**Analysis of copy number variants in 10,377 pregnancies with GGA SNP microarray**

Received: 5 December 2019 | Revised: 23 April 2020 | Accepted: 25 April 2020

DOI: 10.1111/aogs.13886

ORIGINAL RESEARCH ARTICLE

**Detection of copy number variants with chromosomal microarray in 10 377 pregnancies at a single laboratory**Yi-Hui Lin^{1,2} | Yiin-Jeng Jong² | Pin-Chia Huang² | Chris Tsai²

We intended to study the **prevalence of copy number variants (CNVs) in prenatal samples using a single SNP microarray platform** stratified by indication.

From January 2015 to December 2017, a **total of 10,377 prenatal samples were received for prenatal single nucleotide polymorphism (SNP)-microarray** in the laboratory of the Genetics Generation Advancement Corporation. CNVs and region of homozygosity identified by the SNP-microarray were analyzed.

SNP microarray is a preferable chromosomal microarray platform, because it can detect not only CNVs but also uniparental disomy, triploidy, occult trisomy mosaicism and parental origin of CNVs.

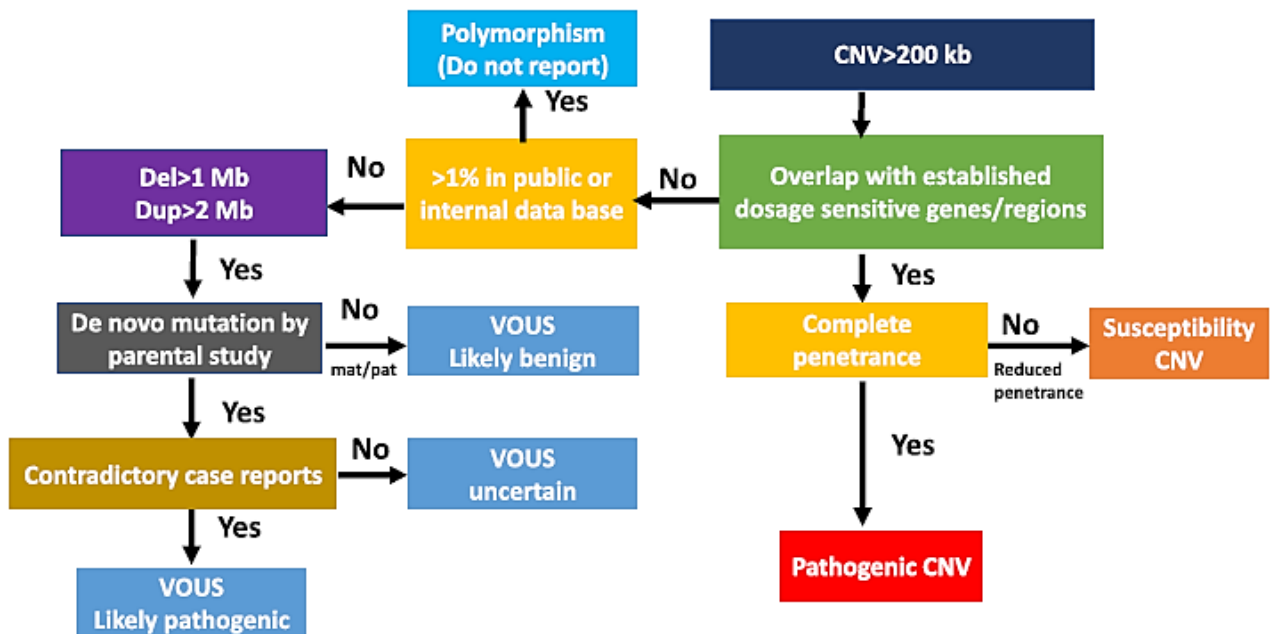
► Indications for chromosomal microarray analysis studies included:

TABLE 1 Sample demographic data (n = 10 377)

Specimen	Case number	
Uncultured amniotic fluid	10 202	
Cultured amniotic fluid cells	162	
Uncultured chorionic villi	10	
Cord blood	3	
Indication	Maternal age (years)	
Advance maternal age	(37.20 ± 2.74)	7704 (74.2%)
Abnormal ultrasound finding	(31.18 ± 3.38)	689 (6.6%)
High risk maternal serum screening	(31.31 ± 2.99)	462 (4.5%)
Anxiety	(31.68 ± 2.91)	1253 (12.1%)
Others ^a	(32.31 ± 4.04)	269 (2.6%)

^aIncluded family history of a genetic condition or chromosome abnormality, a known abnormal fetal karyotype or microarray result.

Classifications and reporting of CNVs in prenatal microarray



Abbreviations: CNV, copy number variants; del, deletion; dup, duplication; mat, maternally inherited; pat, paternally inherited; VOUS, variant of unknown significance.

FIGURE 1 Classifications and reporting of CNVs in prenatal microarray. CNV, copy number variants; del, deletion; dup, duplication; mat, maternally inherited; pat, paternally inherited; VOUS, variant of unknown significance

► CNVs detected with CMA were aligned with known aberrations in public databases :

- ClinGen
- DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources)
- ClinVar
- DGV (Database of Genomic Variants)
- Internal database

► Clinical significance of reported CNV:

- **Pathogenic CNV**
- **Susceptibility CNV (sCNV):**
 - identified as risk factors for neurodevelopmental disorders including autism spectrum disorders, developmental delay, intellectual disability and psychiatric problems.
- **Variants of unknown significance (VOUS):**
 - further divided into likely pathogenic, uncertain and likely benign

Frequency of CNV of CMA analysis for prenatal diagnosis

TABLE 2 Frequency of CNV of CMA analysis in 10 377 samples according to CNV size and indication for prenatal diagnosis

Indications for Prenatal diagnosis	Total no. of cases	CNV n (%)	CNV >5 Mb n (%)	CNV <5 Mb			Total n (%) [95%CI]
				Pathogenic n (%)	sCNV n (%)	VOUS n (%)	
Any	10 377	407 ^a (3.9)	185 (1.8)	42 (0.4)	84 (0.8)	97 (0.9)	223 (2.1) [1.9-2.4]
AMA	7704	263 (3.4)	116 (1.5)	28 (0.4)	57 (0.7)	63 (0.8)	148 (1.9) [1.6-2.2]
AUS	689	53 (7.7)	23 (3.3)	7 (1.0)	11 (1.6)	12 (1.8)	30 (4.4) [3.0-6.1]*
MSS	462	28 (6.1)	16 (3.5)	3 (0.7)	4 (0.8)	5 (1.1%)	12 (2.6) [1.5-4.5]
Anxiety	1253	34 (2.7)	12 (0.9)	3 (0.3)	8 (0.6)	11 (0.9%)	22 (1.8) [1.1-2.6]
Other ^b	269	29 (10.8)	18 (6.7)	1 (0.4)	4 (1.5)	6 (2.2%)	11 (4.1) [2.3-7.1]

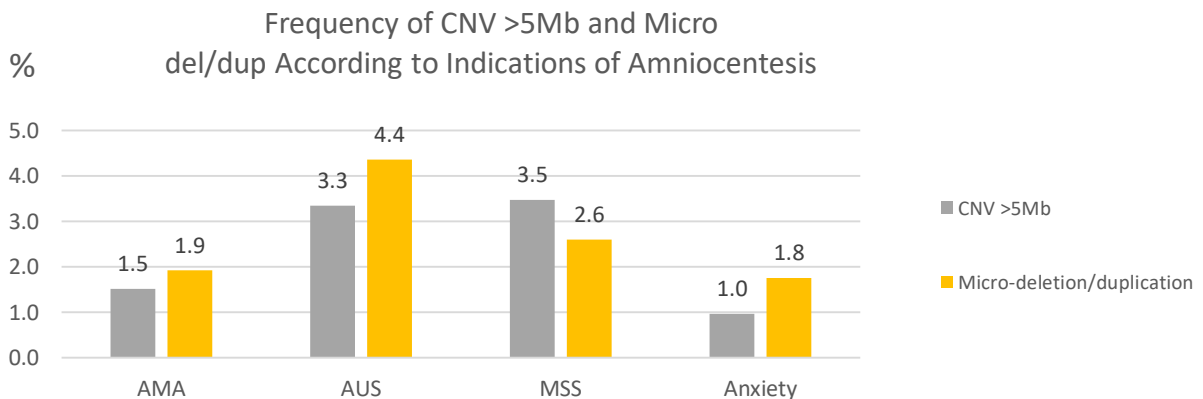
Abbreviations: AMA, advanced maternal age; AUS, abnormal ultrasound finding; CI, confidence interval; CMA, chromosomal microarray; CNV, copy number variant; MSS, high risk of maternal serum screening; sCNV, susceptibility CNV; VOUS, variant of unknown significance.

^aOne fetus carried both karyotypic abnormality (trisomy X) and a CNV.

^bIncluded family history of a genetic condition or chromosome abnormality, a known abnormal fetal karyotype or microarray result.]

*Statistically significant compared with AMA, AUS and anxiety ($P < .001$, Fisher's exact test, 2-sided test).

- ▶ **More than half** (223/407, 55%) of the abnormal results were undetectable by conventional karyotyping.
- ▶ **More than half** (126/223, 57%) of the identified <5Mb CNVs are associated with known clinical phenotypes reported in multiple peer-reviewed publications.
- ▶ **The frequency of VOUS** in this study is 0.9% (97/10377). This rate is similar to previous study using traditional CGH array.



*AMA: advanced maternal age; AUS: abnormal ultrasound finding; MSS: high risk of maternal serum screening

Recurrent pathogenic CNV and sCNV and the inheritance

TABLE 3 Recurrent pathogenic and susceptibility copy number variants and the inheritance

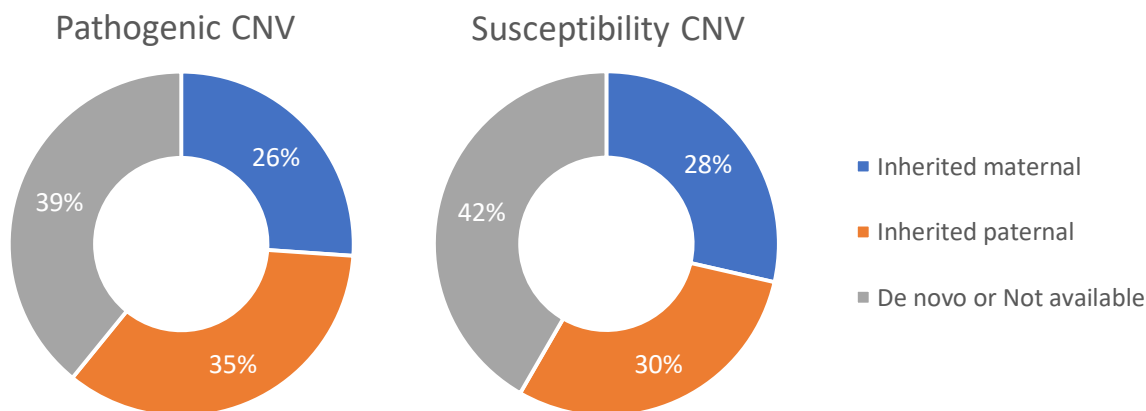
Region (gene within region)			Total no. of cases	Inheritance		
Pathogenic CNV	del/dup	Coordinates arr[hg19]		Inherited mat	pat	De novo or NA
17p12 (PMP22)	dup	17p12(14,000,000_15,400,000)*3	7	4	2	1
17p12 (PMP22)	del	17p12(14,000,000_15,400,000)*1	7	1	5	1 ^a
22q11.2 (TBX6)	del	22q11.2(18,600,000-21,800,000)*1	4	0	0	4
Xp22.31 (STS)	del	Xp22.31(6,400,000-8,100,000)*1	3	1	1	1 ^a
7q11.23	del	7q11.23(72650120_74207565)*1	2	0	0	2
Total			23 (0.2%)	6	8	9
Susceptibility CNV						
1q21.1 (RBM8A)	del	1q21.1(145,300,000_145,700,000)*1	3	0	0	3
Distal 1q21.1 (GJA5)	dup	1q21.1(146,000,000_147,800,000)*3	2	0	0	2
Distal 1q21.1 (GJA5)	del	1q21.1(146,000,000_147,800,000)*1	3	2	1	0
15q11.2 (NIPA1)	del	15q11.2(22,700,000_23,200,000)*1	25	10	7	8 ^a
15q13.3 (CHRNA7)	del	15q13.3(32,000,000_32,400,000)*1	2	2	0	0
16p13.11 (MYH11)	dup	16p13.11(14,800,000_16,500,000)*3	22	6	10	6
16p13.11 (MYH11)	del	16p13.11(14,800,000_16,500,000)*1	9	1	3	5 ^a
Distal 16p11.2 (SH2B1)	del	16p11.2(28,800,000_29,000,000)*1	1	0	0	1
Proximal 16p11.2 (TBX6)	dup	16p11.2(29,500,000_30,100,000)*3	4	1	0	3
Proximal 16p11.2 (TBX6)	del	16p11.2(29,500,000_30,100,000)*1	4	0	0	4
16p13.3 (CREBBP)	dup	16p13.3(3,700,000_3,900,000)*3	1	0	0	1
17q12 (HNF1B)	dup	17q12(34,800,000_36,200,000)*3	2	1	0	1
22q11.2 (TBX1)	dup	22q11.2(18,600,000_21,400,000)*3	6	1	4	1
Total			84 (0.8%)	24	25	35

del/dup, deletion/duplication; NA, not available.

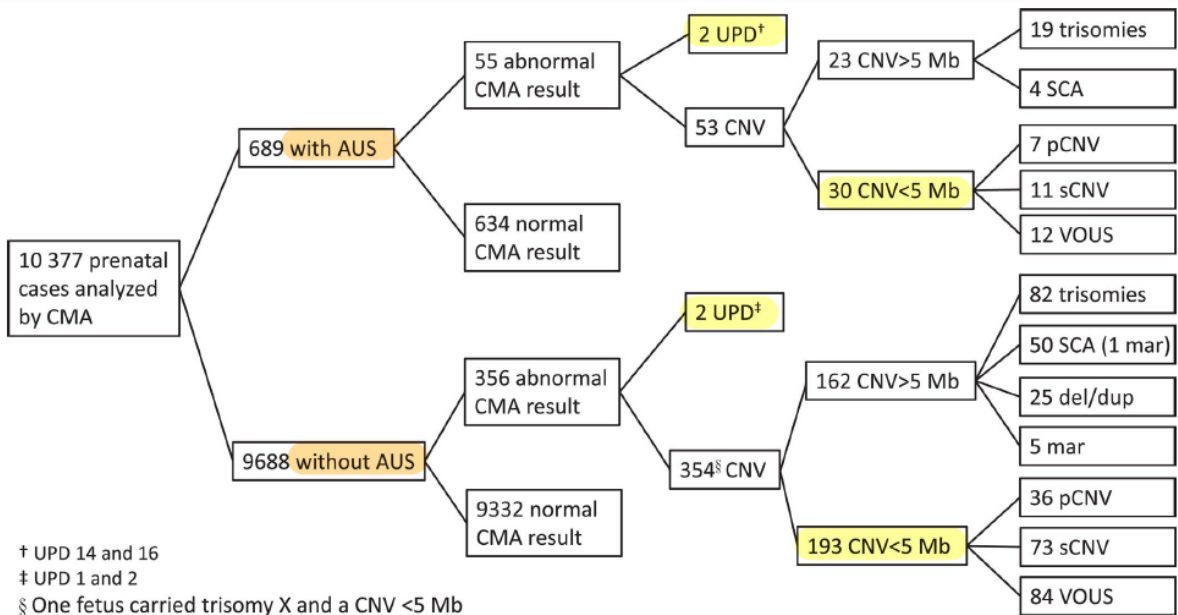
^aThe inheritance of CNV was not available in one case.

► Many CNVs are inherited from unaffected parents. **Parental studies** may provide additional information regarding their inheritance.

► **About 60%** of the pathogenic and susceptibility CNVs are inherited from a parent.



Cytogenomic Profile of cases with vs without Ultrasound Findings



[†] UPD 14 and 16

[‡] UPD 1 and 2

[§] One fetus carried trisomy X and a CNV < 5 Mb

Abbreviations: AUS, abnormal ultrasound finding; CNV, copy number variant; mar, del/dup, deletion/duplication; mar, marker chromosome; pCNV, pathogenic CNV; sCNV, susceptibility CNV; UPD, uniparental disomy; VOUS, variants of unknown significance.

FIGURE 2 Cytogenomic profile of cases with vs without abnormal ultrasound as an indication. [†]UPD 14 and 16. [‡]UPD 1 and 2. [§]One fetus carried trisomy X and a CNV < 5 Mb. AUS, abnormal ultrasound finding; CNV, copy number variant; del/dup, deletion/duplication; mar, marker chromosome; pCNV, pathogenic CNV; sCNV, susceptibility CNV; UPD, uniparental disomy; VOUS, variants of unknown significance

- ▶ **Chromosomal abnormality** was detected in **3.7%** (356/9688) of pregnancies **even without AUS** using SNP microarray.
- ▶ After excluding common and unspecified sonographic findings, **the diagnostic yield of <5Mb CNVs** among fetuses **with AUS** was **6.2%** (14/226), and these CNVs **cannot be detected by conventional karyotyping**.
- ▶ SNP microarray enables the detection of **both CNV and copy number neutral regions of AOH** suggesting the presence of UPD.

Conclusion

- Fetuses carrying **susceptibility CNVs and VOUS** may benefit from **early intervention programs** in which a presymptomatic diagnosis may further improve the outcome.
- **UPD may result from trisomy rescue**, which is often associated with placental or fetal mosaicism, and it **also increase the risk of recessive disorders** of the involved chromosome.
- **SNP microarray can aid in the diagnosis of triploidy, UPD, occult trisomy mosaicism and parental origin of CNVs**, thus improving the interpretation of the results and genetic counseling
- **SNP microarray is a preferable CMA platform** over comparative genomic hybridization-array (a-CGH) in the prenatal diagnosis.
- **Pre- and post-test genetic counseling is critical** for implementing prenatal array testing

GGA SNP microarray test

with its superior sensitivity and resolution is used globally in renowned hospitals and medical centers. It can **EFFICIENTLY detect more chromosomal abnormalities in one single test** because of its unique design with whole genome coverage.

	SNP array		Array CGH (Oligo or BAC)	Karyo- typing
	HD ¹	750K		
Diagnostic resolution ²	Highest	High	Medium	Low
Number of markers	2.69M	750K	60K	X
Number of SNP markers	750K	200K	X	X
Turnaround time	~10 working days			~1 month
Cell culture not required ³ prevent the risk of culture failure and change of the mosaic percentage	○	○	○	X
Identify maternal cell contamination	○	○	X	X
Numerical chromosomal abnormalities leading to Down syndrome, Trisomy 18, Trisomy 13, and etc	○	○	○	○
Micro-deletions/duplications leading to Prader-Willi, Angelman, DiGeorge and William's Syndromes, and etc.	○	○	○	X
Uniparental disomy ⁴ leading to Prader-Willi, Angelman and Beckwith-Wiedemann Syndrome, and etc.	○	○	X	X
Triploidy 69 chromosomes	○	○	X	○
Balanced translocation /inversion/rearrangement	X	X	X	○
Coverage of ClinGen	100%	100%		
Coverage of OMIM Morbid genes	98%	83%		

¹ HD array is similar to FDA-approved chromosomal microarray (CytoScan Dx)

² structural rearrangement and low-level mosaicism of the chromosomes are excluded

³ unless the sample is in low quality or insufficient amount

⁴ uniparental heterodisomy is excluded