Original Article

22q11.2 recurrent copy number variation-related syndrome: a retrospective analysis of our own microarray cohort and a systematic clinical overview of ClinGen curation

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Background: Chromosomal 22q11.2 dosage changes in the recurrent region can lead to a series of clinically variable pediatric syndromes. This study conducted a retrospective analysis of microarray tested cases with 22q11.2 recurrent copy number variations (CNVs) at our laboratory from September 2018 to August 2021, and provides a systematical clinical overview of ClinGen curation.

Methods: The data of 34 microarray tested cases with 22q11.2 recurrent CNVs at our laboratory from September 2018 to August 2021 were retrospectively analyzed, and the variant types, abnormal chromosome regions, clinical phenotypes, and follow-up information were evaluated and summarized. A ClinGen Dosage Sensitivity Map was retrieved for "22q11.2". The information of each 22q11.2 recurrent region was collected and systematically classified.

Results: We reported 34 cases (including 18 22q11.2 microdeletion cases and 16 microduplication cases) from 8,465 microarrays. Of the 22q11.2 recurrent CNV-carried samples, 74% (25/34) comprised prenatal amniotic fluid or villus, and up to 50% (17/34) of the cases contained the proximal A–D interval. Across these 22q11.2 microdeletion samples, the congenital cardiovascular defect, which mainly included the tetralogy of fallot, ventricular septal defect, and patent foramen ovale, was identified as the most common feature (13/18, 72%). However, 22q11.2 microduplication cases exhibited a broad range of highly variable phenotypes, spanning from severe abnormality to mild characteristics and even the completely normal phenotype. This study also systematically reviewed the ClinGen dosage sensitivity curation on 22q11.2 recurrent regions, and found that A–D/A–B haploinsufficiency score reached "3", responsible for DiGeorge syndrome (DGS)/velocardiofacial syndrome (VCFS). Also, A–D/A–B triplosensitivity score "3" could further account for multiple variable phenotypes.

Conclusions: Taken together, this study provides clinical overview of the ClinGen curation and data support for the American College of Medical Genetics and Genomics (ACMG) evaluation in the pathogenicity of each interval involved in 22q11.2 recurrent deletion and duplication. Certainly, more evidences on the genotype-phenotype contributions of different 22q11.2 recurrent CNVs need to be gathered.

Keywords: 22q11.2 recurrent copy number variations (22q11.2 recurrent CNVs); retrospective analysis; overview; ClinGen curation

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Introduction

A cluster of low-copy repeats (LCRs) from A–H in chromosome 22q11.2 (chr22:17,900,001–25,900,000), also known as LCR22A–H, mediate nonallelic homologous recombination and cause 22q11.2 chromosomal rearrangements. Various intervals occur during this process that are respectively described as "proximal," "central," and "distal" on the basis of the corresponding length and region of the recurrent copy number variations (CNVs) (1,2). Both the recurrent regions [(i.e., 3 Mb (A–D: chr22:18,912,231–21,465,672) and 1.5 Mb (A–B: chr22:18,912,231–20,287,208)] included in the proximal CNVs are the most common types for 22q11.2 microdeletions (the small deleted CNVs) or microduplications (the small duplicated CNVs). As previously reported, the proximal A–D or A–B deletion may lead to severe DiGeorge syndrome (DGS)/velocardiofacial syndrome (VCFS) (3), while identical duplication results in highly variable and nonspecific phenotypes (4).

The 22q11.2 central and distal CNV syndromes also express phenotypic variability, spanning from severe abnormality to mild characteristics and even a completely normal phenotype (5,6). This creates great challenges for genetic counseling and the prediction of clinical consequences. Just recently, the prenatal ultrasound phenotypes of CNVs in different regions of 22q11.2, their parental original, as well as pregnancy outcome, were analyzed and reported by Peixuan group. Interestingly, they found that prenatal phenotypes of the 22q11.2 region CNVs are diverse, which may be related to gene function; nuchal translucency (NT) thickening may be used as an early ultrasound finding of proximal 22q11.2 CNV (7). In this study, we retrospectively analyzed 34 microarray cases with 22q11.2 recurrent CNVs at our own laboratory from September 2018 to August 2021, and systematically reviewed each recurrent 22q11.2 region for which the ClinGen expert group had completed curation (derived from the ClinGen Dosage Sensitivity Map that ClinGen consortium curates genes and regions of the genome to assess whether there is evidence to support that these genes/regions are dosage sensitive and should be targeted on a cytogenomic array). We present the following article in accordance with the STROBE reporting checklist (available at https://dx.doi.org/10.21037/tp-21-560).

Methods

Collection of clinical information

We undertook a retrospective analysis of 34 cases of 22q11.2 recurrent CNVs (including 18 22q11.2 microdeletion cases and 16 microduplication cases) from 8465 microarrays performed at our own laboratory from September 2018 to August 2021. Prenatal amniotic fluid and villus, abortion tissue and villus, as well as peripheral blood derived from children/adults were used in our analysis. Data on gestation/age, the chromosome microarray analysis (CMA) results and corresponding variant evaluation types, clinical phenotypes (prenatal samples conforming to B ultrasound data), and tests were collected and are summarized in *Table 1*. After post-test counseling, a fraction of parents received a CMA analysis to validate their fetuses' hereditary mode. Additionally, 5 months after CMA testing, the pregnant women and probands were followed up with by telephone. All the available information is displayed in *Table 1*. "N/A" represents a loss of communication, an unwillingness to inform, or an unknown hereditary mode. Written informed consent was provided by each pregnant woman, proband, or their parents. The study was approved by ethics board of Ningbo Women and Children's Hospital (No. EC2020-014). This study conformed to the provisions of the Declaration of Helsinki (as revised in 2013).

CMA

After samples were collected, DNA was extracted using a TIANamp Genomic DNA Kit (Cat.#DP304-02; Lot#U8420). Then, 250ng DNA was amplified, labeled, and hybridized to the GCS 3000Dx v.2 platform (Affymetrix, Santa Clara, CA, USA). The SNP array test was processed with a commercial 750K microarray chip (CytoScan 750K Array; Affymetrix). The chip was washed with buffer, scanned with a laser scanner after hybridization with fragmented DNA. The data were analyzed using Chromosome Analysis Suite v3.2 (Affymetrix).

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Table 1 The clinical information of 34 cases carried with 22q11.2 recurrent CNVs

22q11.2 CNV type	Case number	Sample type	Gestation/ age	CMA analysis result	Variant evaluation type	22q11.21 recurrent region	Clinical phenotypes and tests (contain ultrasound data)	Follow-up information	Parental validation (yes/not)	Hereditary mode
Microdeletion	1	Amniotic fluid	20W + 6D	arr[hg19] 22q11.21(20,716,876–21,800,471) ×1	Likely pathogenic	Contain central (B–D) region	NIPT: a microdeletion of chromosome 22; B ultrasound: strong light spots in the left ventricle, patent foramen ovale, oligohydramnios	Premature infant, anemiaofprematurity, neonatal respiratory distress syndrome, neonatal pneumonia, low birth weight infant	No	N/A
	2		20W	arr[hg19] 22q11.22q11.23(22,997,928–23,654,007) ×1	Likely pathogenic	Contain distal type II (E–F) region	Advanced maternal age	N/A	Yes	Inherited from mother
	3		25W + 2D	arr[hg19] 22q11.21(20,716,876–21,800,471) ×1	Pathogenic	Contain central (B–D) region	NIPT: a local microdeletion of chromosome 22; B ultrasound: patent foramen ovale	N/A	Yes	Inherited from mother
	4		26W + 3D	arr[hg19] 22q11.21(18,916,842–21,800,471) ×1	Pathogenic	Contain proximal (A–D) region	B ultrasound: congenital heart disease (perimembrane ventricular septal defect)	N/A	Yes	*De novo*
	5		19W + 2D	arr[hg19] 22q11.21(18,916,842–21,800,471) ×1	Pathogenic	Contain proximal (A–D) region	The histories of abnormal pregnancy: gave birth to a child with tetralogy of fallot; B ultrasound: complicated congenital heart disease (tetralogy of fallot + pulmonary atresia), the absent thymus	N/A	Yes	Inherited from mother
	6		19W + 6D	arr[hg19] 22q11.21(20,716,876–21,800,471) ×1	VUS	Contain central (B–D) region	NIPT: a microdeletion of chromosome 22	N/A	Yes	Inherited from mother
	7		20W	arr[hg19] 22q11.21(18,919,477–21,800,471) ×1	Pathogenic	Contain proximal (A–D) region	Advanced maternal age; NIPT: a microdeletion of chromosome 22; B ultrasound: complicated congenital heart disease (tetralogy of fallot)	N/A	No	N/A
	8		27W	arr[hg19] 22q11.21(18,919,477–21,800,471) ×1	Pathogenic	Contain proximal (A–D) region	NIPT: 5 Mb deletion in 22q11.1q11.21; B ultrasound: fetal vagus right subclavian artery	N/A	Yes	*De novo*
	9		19W + 2D	arr[hg19] 22q11.21(20,730,144–21,800,471) ×1	VUS	Contain central (B–D) region	NIPT: a microdeletion of chromosome 22	N/A	Yes	Inherited from mother
	10		25W	arr[hg19] 22q11.21(18,919,478–21,058,888) ×1	Pathogenic	Overlap with proximal (A–D) region	B ultrasound: right aortic arch, strong light spot in right ventricle, slightly enlarged right atrium	Embryo arrest at 28W	Yes	*De novo*
	11		20W + 4D	arr[hg19] 22q11.21q11.22(21,917,140–22,962,962) ×1	Pathogenic	Overlap with distal type I (D–E/F)	NIPT suggested microdeletion on chromosome 22	N/A	No	N/A
	12	Peripheral blood	10Y	arr[hg19] 22q11.21(18,919,477–21,800,471) ×1	Pathogenic	Contain proximal (A–D) region	Multiple malformations of abnormal sexual development (micropenis, microrchidia), intellectual/physical retardation and congenital heart disease	N/A	No	N/A
	13	Abortion tissue	23W	arr[hg19] 22q11.21(18,648,855–21,915,207) ×1	Pathogenic	Contain proximal (A–D) region	B ultrasound: the possible pulmonary dysplasia with atresia and severe stenosis, severe tetralogy of fallot	Odinopoeia	No	N/A
	14		22W + 3D	arr[hg19] 22q11.21(19,024,793–21,800,471) ×1	Pathogenic	Contain proximal (A–D) region	B ultrasound: persistent truncus arteriosus, ventricular septal defect (malalignment type)	Odinopoeia	Yes	*De novo*
	15		20W + 4D	arr[hg19] 22q11.21(18,648,855–21,800,471) ×1	Pathogenic	Contain proximal (A–D) region	B ultrasound: ventricular septal defect, aortic riding, pulmonary stenosis, suggesting tetralogy of fallot; PLSVC	Odinopoeia	Yes	*De novo*
	16		17W + 2D	arr[hg19] 3q26.1(161,044,139–161,888,507) ×3; arr[hg19] 22q11.21(21,058,887–21,800,471) ×1	Pathogenic	Overlap with proximal (A–D) region	B ultrasound: ventricular septal defect, aortic riding, pulmonary stenosis, suggesting tetralogy of fallot; PLSVC	Odinopoeia	No	N/A
	17		24W	arr[hg19] 22q11.21(18,648,856–21,800,471) ×1	Pathogenic	Overlap with proximal (A–D) region	Congenital heart disease, left kidney dysplasia	N/A	No	N/A
	18	Abortion villus	13W	arr[hg19] 22q11.21(18,919,478–21,800,471) ×1	Pathogenic	Overlap with proximal (A–D) region	NT thickening; lymphatic hydrocystic tumor	N/A	No	N/A

Table 1 (*continued*)

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Table 1 (*continued*)

22q11.2 CNV type	Case number	Sample type	Gestation/ age	CMA analysis result	Variant evaluation type	22q11.21 recurrent region	Clinical phenotypes and tests (contain ultrasound data)	Follow-up information	Parental validation (yes/not)	Hereditary mode
Microduplication	19	Amniotic fluid	19W + 4D	arr[hg19] 22q11.21(18,648,855–21,800,471) ×3	Pathogenic	Contain proximal (A–D) region	The abnormal prenatal BoBs result: 22q11.2 microduplication	N/A	No	N/A
	20		22W + 1D	arr[hg19] 7q11.23(72,624,166–74,197,150) ×1; arr[hg19] 22q11.21(18,648,855–21,800,471) ×3	Pathogenic	Contain proximal (A–D) region	Advanced maternal age; B ultrasound: ventricular septal defect, the increased S/D ratio of umbilical artery blood flow, undetected right kidney	Postpartum neonatal jaundice	No	N/A
	21		21W + 5D	arr[hg19] 22q11.21(18,970,561–21,800,471) ×3	Pathogenic	Contain proximal (A–D) region	Fetal serological screening: the high risk of 21 trisomy	N/A	Yes	*De novo*
	22		21W + 4D	arr[hg19] 22q11.21(18,648,855–21,459,713) ×3	Pathogenic	Contain proximal (A–D) region	NIPT: a local microduplication of chromosome 22; B ultrasound: oligohydramnios	N/A	No	N/A
	23		26W + 6D	arr[hg19] 22q11.21(18,648,855–21,800,471) ×3	Pathogenic	Contain proximal (A–D) region	B ultrasound: fetal cerebral ventriculomegaly: 11 mm	N/A	Yes	Inherited from father
	24		19W + 4D	arr[hg19] 22q11.21(20,716,902–21,461,017) ×3	VUS	Contain central (B–D) region	Fetal serological screening: the high risk of 21 trisomy; NT thickening: 2.8 mm	N/A	No	N/A
	25		19W + 4D	arr[hg19] 22q11.23(23,692,307–24,987,835) ×3	VUS	Contain distal type III (F–G) region	Fetal serological screening: the high risk of 21 trisomy	N/A	No	N/A
	26		20W + 1D	arr[hg19] 22q11.21(18,919,477–21,800,471) ×3	Pathogenic	Contain proximal (A–D) region	Advanced maternal age; the pregnant woman with macular degeneration and neurodeatrophia; B ultrasound: oligohydramnios	Postpartum B ultrasound: patent foramen ovale; neonatal dyspnea syndrome; neonatal pneumonia	No	N/A
	27		24W	arr[hg19] 22q11.21(18,916,960–21,800,471) ×3	Pathogenic	Contain proximal (A–D) region	The histories of abnormal pregnancy: gave birth to a retarded child with her ex-husband; B ultrasound: a fissure is seen in the gall bladder, strong light spots in the left ventricle	N/A	No	N/A
	28		19W + 2D	arr[hg19] 6q22.31(124,612,649–125,958,277) ×3; arr[hg19] 22q11.22q11.23(22,997,928–23,652,586) ×3	Likely Benign	Contain distal type II (E–F) region	The histories of abnormal pregnancy: labor induction for long bone dysplasia; abnormal 6q22.31, 22q11.22q11.23 in the first fetus and the mother	N/A	Yes	Inherited from mother
	29		20W	arr[hg19] 22q11.21(18,648,855–21,800,471) ×3	Pathogenic	Contain proximal (A–D) region	Fetal serological screening: the high risk of 21 trisomy	N/A	No	N/A
	30		21W + 2D	arr[hg19] 22q11.21(18,916,842–21,800,471) ×3	Pathogenic	Contain proximal (A–D) region	Fetal serological screening: the high risk of 21 trisomy; B ultrasound: the small and obscured transparent diaphragmatic cavity	N/A	No	N/A
	31		20W + 4D	arr[hg19] 22q11.21(18,919,478–21,800,471) ×3	Pathogenic	Overlap with proximal (A–D) region	Fetal serological screening at high risk, DS:1/236	N/A	No	N/A
	32	Prenatal fluff	12W + 3D	arr[hg19] 22q11.21(18,648,856–21,800,471) ×3	Pathogenic	Overlap with proximal (A–D) region	NT thickening: 3.7 mm; embryo stop once	N/A	No	N/A
	33	Peripheral blood	30Y	arr[hg19] 22q11.23(23,652,586–25,059,827) ×3	VUS	Contain distal type III (F–G) region	N/A	N/A	No	N/A
	34	Abortion tissue	25W + 3D	arr[hg19] 6q22.31(124,612,649–125,928,351) ×3; arr[hg19] 22q11.22q11.23(22,997,928–23,650,873) ×3	VUS	Contain distal type II (E–F) region	The previous microarry result of aborted fetal tissue: arr[hg19] 6q22.31(124,612,649-125,928,351) ×3; arr[hg19] 22q11.2 2q11.23(22,997,928-23,650,873) ×3; B ultrasound: short limbs long-bone, slightly weakened echo of spine and other whole body bone	N/A	Yes	Inherited from mother

CNVs, copy number variations; W, weeks; D, days; Y, years; NIPT, noninvasive prenatal testing; NT, nuchal translucency; VUS, variant of uncertain significance; PLSVC, perpetuate left superior vena cava; S/D, systolic/diastolic.

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Overview of ClinGen curation

A ClinGen Dosage Sensitivity Map was retrieved for "22q11.2". The information of each 22q11.2 recurrent region was collected. The score of each interval's haploinsufficiency/triplosensitivity in current ClinGen curation, and respective clinical features, hereditary mode, and penetrance were all systematically classified and are summarized in *Table 2*.

Statistical analysis

The data were analyzed using Microsoft Excel 2010, and not involved in complex statistical analysis.

Results

Case presentation of 22q11.2 recurrent CNVs

We performed a retrospective analysis of 8,465 microarray cases at our laboratory from September 2018 to August 2021. We identified 34 patients carrying 22q11.2 recurrent CNVs (including 18 microdeletion cases and 16 microduplication cases), and examined their genotype-phenotype correlations. Their detailed clinical information is summarized in *Table 1*. Similar to previous reports, the 22q11.2 CNVs containing the proximal A–D interval comprised the most common recurrent region in our CMA data (17/34, 50%). In addition, the aberrant 22q11.2 CNVs of the other 6 cases (including 4 deleted fragments and 2 duplicated fragments) overlapped with the A–D region (6/34, 17.6%). As expected, all of these 22q11.2 CNVs were assessed as pathogenic variants. The central B–D interval (chr22:20,731,986–21,465,672) was found to be involved in the deletion/duplication regions of 5 cases (5/34, 14.7%). Additionally, 6 distal 22q11.2 CNVs were found to contain type II (E–F) (3/34, 8.8%) and type III (F–G) (2/34, 5.9%) regions and overlap with the type I (D–E/F) (1/34, 2.9%) interval. Notably, partially due to their variable phenotypes, incomplete penetrance, low dosage, or incomplete sensitivity curation, the majority of these CNVs were evaluated as variants of uncertain significance according to the American College of Medical Genetics and Genomics (ACMG) criteria.

Our cases were also accompanied by clinical phenotypes (ultrasound data), fractional follow-up information, and hereditary mode data. Across these 22q11.2 microdeletion samples, the congenital cardiovascular defect was identified as the most common feature (13/18, 72%), and mainly included tetralogy of fallot, ventricular septal defect, and patent foramen ovale. In particular, the pathogenic microdeletion variants concerning these congenital heart diseases generally spanned the proximal A–D interval. Most of the samples involved comprised prenatal amniotic fluid, so the clinical characteristics of the fetus were mainly distinguished by a B ultrasound combined with postnatal follow-up data. First, by detection with amniotic fluid, we exemplified cases 1 and 5. In the prenatal B ultrasound of the case 1 fetus with central B–D deletion, we observed strong light spots in the left ventricle and a patent foramen ovale. The postnatal follow-up information revealed more severe features, including a premature infant, anemia of prematurity, neonatal respiratory distress syndrome, neonatal pneumonia, and a low birth weight. In case 5, the pregnant woman had ever before given birth to an abnormal child with tetralogy of fallot. Unfortunately, the B ultrasound of the case 5 fetus indicated a more complicated congenital heart disease (tetralogy of fallot and pulmonary atresia) combined with an absent thymus, but the validated parental result suggested that the proximal A–D CNV of case 5 was inherited from the mother, despite the fact that >90% A–D deleted cases are *de novo* (3).

Among the 18 microdeletion cases, case 12 was a 10-year-old child who was confirmed to carry a pathogenic A–D deletion that was responsible for his multiple malformations of abnormal sexual development (i.e., a micropenis and microrchidia), intellectual/physical retardation, and congenital heart disease. Except for amniotic fluid and peripheral blood detections, the microdeletion CMA data of the abortion tissues and abortion villi are also set out in Table 1. As we observed, cases 13–15 contained the proximal (A–D) region and cases 16–17 overlapped with the proximal (A–D) region, and all of these cases presented with complicated congenital heart disease, including a severe tetralogy of fallot, ventricular septal defect, persistent truncus arteriosus, or perpetuate left superior vena cava (PLSVC). Parental validation of cases 14 and 15 indicated *de novo* variants.

Consistent with ClinGen triplosensitivity phenotype comments, the 22q11.2 recurrent microduplications exhibited a broad range of highly variable phenotypes. Of these affected amniotic fluid cases, 46% of the pregnant women primarily sought CMA help due to the high risk of 21 trisomy in fetal serological screening. The B ultrasound results revealed multiple variable phenotypes, including a ventricular septal defect, undetected right kidney, fetal cerebral ventriculomegaly, fissure in the gall bladder, and small and obscured transparent diaphragmatic

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Table 2 The systematical clinical overview of ClinGen curation on 22q11.2 recurrent CNVs

22q11.21 recurrent region	Overlap	Contained							
		Proximal		Central		Distal			
		A–B	A–D	B–D	C–D	Type I (D–E/F)	Type II (E–F)	Type III (D–H)	Type III (F–G)
Region location (GRCh37)	chr22:17,392,953–18,591,860	chr22:18,912,231–20,287,208	chr22:18,912,231–21,465,672	chr22:20,731,986–21,465,672	chr22:21,092,338–21,465,672	chr22:21,917,117–23,649,111	chr22:23,119,414–23,649,111	chr22:21,917,117–24,994,433	chr22:23,831,202–24,632,821
Key morbid genes	*CECR2*	*TBX1*		*CRKL*		N/A	N/A	*SMARCB1*	
The score of dosage sensitivity in ClinGen gene curation									
Haploinsufficiency	0	3	3	2	N/A	3	N/A	N/A	N/A
Triplosensitivity	3	3	3	1	N/A	3	N/A	N/A	N/A
Clinical features									
Haploinsufficiency	Rare in both the literature and databases of genomic variation (both clinical and control populations)	DGS/VCFS syndrome, congenital heart disease, palatal abnormalities, characteristic facial features, DD/ID, behavior problems, immune deficiency, hypocalcemia		Phenotypic variability include: dysmorphic facial features, growth restriction/short stature, CNS anomalies/seizures, developmental delay (including language delay), intellectual disability, psychiatric/ behavioral problems, skeletal anomalies, cardiovascular defects, genitourinary anomalies, and immune deficiency/recurrent infections	N/A	Phenotypic variability include: preterm birth, pre- and/or postnatal growth restriction, DD/ID, behavioral problems, cardiovascular defects, skeletal anomalies and mild dysmorphic facial features	N/A	N/A	N/A
Triplosensitivity	CES, phenotypic variability	Highly variable clinical phenotype, ranging from apparently normal to expression a broad range of clinical features, including nonspecific phenotypes or phenotypes that overlap clinical findings of DGS/VCFS		Phenotypic variability	N/A	Phenotypic variability include: developmental delays and facial dysmorphisms	N/A	N/A	N/A
Hereditary mode									
Haploinsufficiency	N/A	>90% are *de novo*		60% are *de novo*	N/A	The majority are *de novo*	N/A	N/A	N/A
Triplosensitivity	N/A	Frequently inherited		N/A	N/A	N/A	N/A	N/A	N/A
Penetrance									
Haploinsufficiency	N/A	Enriched in the clinical population		Incomplete	N/A	Enriched in the clinical population	N/A	N/A	N/A
Triplosensitivity	N/A	Incomplete, enriched in the clinical population		Incomplete	N/A	Incomplete, enriched in the clinical population	N/A	N/A	N/A

CNVs, copy number variations; CES, cat eye syndrome; DGS/VCFS, DiGeorge syndrome/velocardiofacial syndrome; DD/ID, developmental delay/intellectual disability; CNS, central nervous system.

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cavity. Another abortion tissue with a duplicated variant of uncertain significance (VUS) inherited from the mother contained a distal type II (E–F) region, and presented with shorter long bones of the limbs, a slightly weakened echo of the spine and other body bones.

Clinical overview of the ClinGen curation

The proximal A–D/A–B recurrent CNVs are responsible for the most common microdeletions or microduplications in chromosome 22q11.2. In particular, A–D/A–B regional deletions can lead to severe DGS/VCFS syndrome for which clinical phenotypes typically include congenital heart disease (particularly conotruncal malformations), palatal abnormalities (particularly velopharyngeal incompetence, cleft palate, and bifid uvula), characteristic facial features, developmental delay and intellectual disability (DD/ID), behavior problems, immune deficiency, and hypocalcemia (3,8). The score of haploinsufficiency sensitivity in the ClinGen gene curation reached "3". Among the 30 involved protein coding genes, *TBX1* was identified as the most crucial morbid gene for DGS/VCFS syndrome. Yagi *et al*. found that a heterozygous 1 base pair deletion (1223delC) in the *TBX1* gene caused a frameshift leading to a stop codon, and thus induced conotruncal anomaly face syndrome/VCFS (9). Paylor *et al*. reported that a heterozygous 23 base pair deletion (1320–1342del23) in the *TBX1* gene could result in a frameshift and the extension of the protein from 504 to 616 amino acids, for which a mutation was found in a mother and her 2 sons who presented with VCFS (10). Nevertheless, patients with proximal duplication in 22q11.2 share a more variable clinical phenotype, ranging from apparently normal to nonspecific phenotypes (e.g., intellectual disability, learning disability, developmental delays, autism, psychiatric disorder growth delays, and hypotonia) (2,4). According to the literature, >90% 22q11.2 proximal (DGS/VCFS) deletions are *de novo* (3), while A–B/A–D duplications are frequently inherited (4). Notably, cat eye syndrome (CES) results from the tetrasomy/triplication of the CES critical region (CESCR) that overlaps with the chromosome 22q11.2's proximal region. The CESCR includes the *CECR1* and *CECR2* genes, which are responsible for heart/facial and neurologic/eye features, respectively, but does not involve the DGS/VCFS syndrome region of 22q11.2 (11,12).

22q11.2 central recurrent CNVs contain B–D and C–D regions. The current haploinsufficiency score of B–D reaches "2" for the emerging evidence of phenotypic variability, including dysmorphic facial features, growth restriction/short stature, central nervous system (CNS) anomalies/seizures, developmental delay (including language delay), intellectual disability, psychiatric/behavioral problems, skeletal anomalies, cardiovascular defects, genitourinary anomalies, and immune deficiency/recurrent infections. Among the collected cases, 60% of the deletions were confirmed to be *de novo* events by parental testing (1,5,13). Similarly, the current triplosensitivity score for the B–D region is only "1" due to the limited number of patients reported in the literature, phenotypic variability, incomplete penetrance, and the lack of case-controlled data.

The distal CNVs of 22q11.2 mainly include 3 types, and currently, only the haploinsufficiency and triplosensitivity of type I (D–E/F) have been curated. ClinGen experts summarized the variable clinical phenotypes of D–E/F deletion: preterm birth, pre- and/or postnatal growth restriction, DD/ID, behavioral problems, cardiovascular defects, skeletal anomalies, and mild dysmorphic facial features. The deletions are *de novo* events in the majority of cases, but occasionally a few carrier parents are reported to have a mild or normal phenotype (14,15). Conversely, the variable clinical phenotypes of D–E/F microduplication mainly include developmental delays and facial dysmorphisms that exhibit incomplete penetrance in the clinical population (16,17). The above contents are included in the ClinGen dosage sensitivity curation page, and have been further summarized in *Table 2*. The dosage sensitivity curation of type II (E–F), type III (D–H or F–G) CNVs have not been completed, but sporadic cases have been reported.

Discussion

Multiple studies have conducted systematic reviews of 22q11.2 microdeletion/microduplication syndrome, performed statistical analyses of their own CMA data and elucidated possible genotype-phenotype contributions (18-20). For example, Pinchefsky *et al*. reported on the clinical phenotype and cytogenetic studies of a 3-year-old girl with a *de novo* distal 22q11.2 duplication, reviewed the literature associated with distal 22q11.2 duplication, and compared the clinical features of 28 previously published cases (6). Burnside's review article on *Gytogenetic and Genome Research* systematically classified each the CNV of 22q11.2 region and provided a detailed overview of patients' clinical features (as available in the literature). Additionally, their own cohort of postnatal and prenatal microarray cases

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with the 22q11.2 CNVs abnormality were also included in their review (1). Emerging reports of patients with 22q11.2 variants in the literature provide favorable evidence for the ClinGen dosage sensitivity curation experts, who are curating genes and regions of the genome to assess whether there is evidence that these genes/regions are dosage sensitive. The score of haploinsufficiency or triplosensitivity in ClinGen gene curation could further support the pathogenicity evaluation of chromosome CNVs.

According to the ClinGen dosage sensitivity curation and clinical overview of 22q11.2 recurrent CNVs, we learned that patients with 22q11.2 microdeletion/microduplication syndrome presented with a wide range of phenotypes concerning the abnormality of multiple systems, of which congenital heart diseases and facial dysmorphisms are the most common congenital malformations. Data on the penetrance of these recurrent CNVs is highly incomplete; some patients appear phenotypically normal, while others with the same genotype have mild to severe abnormalities. Thus, more evidence on the genotype-phenotype contributions of different 22q11.2 deleted/duplicated regions needs to be gathered.

In our summary of the abnormal 22q11.2 CNV cohort, the variants associated with proximal (A–D) region showed more severe clinical phenotypes, while those associated with the central (B–D) and distal type I (D–E/F)/type II (E–F)/type III (F–G) regions exhibited more mild or even normal features. In our laboratory, the majority of microarray cases used prenatal amniotic fluid. Due to the pathogenicity, potential terrible ending for the child, huge financial and psychological burden, most pregnant women choose odinopoeia, and only a fraction eventually give birth to their children. However, the postnatal newborns displayed various abnormalities, including neonatal dyspnea syndrome, and neonatal pneumonia. Among the 14 cases that received parental validation, 6 cases had *de novo* variants (6/14, 42.9%) and the other 8 were inherited from their parents (8/14, 57.1%) (7 of the 8 from the mother). In conclusion, this study provides clinical overview of the ClinGen curation and data support for ACMG evaluation of the pathogenicity of each interval involved in 22q11.2 recurrent deleted and duplicated CNVs. Certainly, more evidences on the genotype-phenotype contributions of different 22q11.2 recurrent regions need to be gathered.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work, including ensuring that questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved. Written informed consent was provided by each pregnant woman, proband, or their parents. The study was approved by ethics board of Ningbo Women and Children's Hospital (No. EC2020-014). This study conformed to the provisions of the Declaration of Helsinki (as revised in 2013).

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