

The recurrent distal 22q11.2 microdeletions are often de novo and do not represent a single clinical entity: a proposed categorization system

Fady M. Mikhail, MD, PhD¹, Rachel D. Burnside, PhD², Brooke Rush, MS², Jennifer Ibrahim, MD³, Robin Godshalk, MS³, S. Lane Rutledge, MD¹, Nathaniel H. Robin, MD¹, Maria D. Descartes, MD¹ and Andrew J. Carroll, PhD¹

Purpose: The five segmental duplications (LCR22-D to -H) at the distal region of chromosome 22 band q11.2 in the region immediately distal to the DiGeorge/velocardiofacial syndrome deleted region have been implicated in the recurrent distal 22q11.2 microdeletions. To date, the distal 22q11.2 microdeletions have been grouped together as a single clinical entity despite the fact that these deletions are variable in size and position depending on the mediating LCR22s.

Methods: Here, we report 13 new unrelated patients with variable size deletions in the distal 22q11.2 region as shown by cytogenomic array analyses. We compare our patients' clinical features with those of previously reported cases to better dissect the phenotypic correlations based on the deletion size and position.

Results: Six patients had the 1.1-Mb deletion flanked by LCR22-D and -E, and presented clinically with a phenotype consistent with

previously reported cases with distal 22q11.2 microdeletions. Three patients had the 1.8-Mb deletion flanked by LCR22-D and -F, and presented with a similar phenotype. Four patients had the 700-kb deletion flanked by LCR22-E and -F, and presented with a milder phenotype that lacked growth restriction and cardiovascular defects.

Conclusion: We suggest that the recurrent distal 22q11.2 microdeletions do not represent a single clinical entity, and propose categorizing these deletions into three types according to their genomic position. All three deletion types are thought to be pathogenic and are most often de novo. They all share some presenting features but also have their unique features and risks.

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Key Words: CNVs; distal 22q11.2 microdeletion; LCR22s; rhabdoid tumors; *SMARCB1*

INTRODUCTION

Recurrent microdeletions and microduplications, also termed recurrent copy-number variations (CNVs), have been identified as a common cause of developmental delay (DD), intellectual disability (ID), autism spectrum disorders, other neuropsychiatric phenotypes, and/or multiple congenital anomalies.¹⁻³ The great majority of these recurrent CNVs are mediated by recombination between nonallelic homologous segmental duplications, also called low copy repeats (LCRs), through the well-established mechanism of nonallelic homologous recombination.⁴ In recent years, the detection of these recurrent events has been facilitated by the wide use of cytogenomic arrays in clinical diagnostics. Certain regions of the human genome are enriched with multiple LCR clusters that contain several modules with a very high degree of sequence homology (>95%), which results in genomic instability of these regions.³ Examples include 1q21.1, 7q11.23, 8p23.1, 15q11.2q13.1, 15q13.3, 16p13.11, 16p11.2, 17p12, 17p11.2, 17q11.2, 17q21.31, and 22q11.2.

Eight LCR clusters have been identified in the proximal region of 22q at band q11.2, and have been named LCR22-A to H. The four centromeric LCR22s (LCR22-A to D) are implicated in the recurrent DiGeorge/velocardiofacial syndrome (DG/VCFs)

microdeletion (OMIM 188400 and 192430), and its reciprocal microduplication (OMIM 608363). Based on the mediating LCR22s, about 90% of patients with the DG/VCFs microdeletion have the common ~3.0 Mb deletion between LCR22-A and D, whereas ~7% of the patients have a smaller nested ~1.5 Mb deletion between LCR22-A and B.⁵ These microdeletions in the proximal portion of the 22q11.2 region are the most common recurrent, pathogenic microdeletions in humans with a frequency of approximately 1:4,000 to 1:8,000 live births.⁶

Recently, others and we demonstrated that the five telomeric LCR22s, namely LCR22-D to H, at the distal portion of 22q11.2 are causally implicated in the recurrent distal 22q11.2 microdeletion-associated phenotype(s) (OMIM 611867), and their reciprocal microduplications in the region immediately distal to the DG/VCFs typically deleted region (Figure 2).⁷⁻¹⁸ The structure of the LCR22-D, -E, and -F has been studied and was shown to contain the *BCRL* module in each LCR22, suggesting that the distal 22q11.2 microdeletions/microduplications are mediated by nonallelic homologous recombination.¹⁰

To date, the distal 22q11.2 microdeletions have been grouped together as a single clinical entity despite the fact that these deletions are variable in size and position depending on the

¹Department of Genetics, University of Alabama at Birmingham, Birmingham, Alabama, USA; ²Laboratory Corporation of America, Research Triangle Park, North Carolina, USA;

³Department of Pediatrics, Division of Genetics, St. Joseph's Children's Hospital, Paterson, New Jersey, USA. Correspondence: Fady M. Mikhail (fmikhail@uab.edu)

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Table 1 Clinical and molecular cytogenetic features of our patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13
LCR22s involved	D-E	D-E	D-E	D-E	D-E	D-E	D-F	D-F	D-F	E-F	E-F	E-F	E-F
FISH probe	RP11-647D11	RP11-647D11	RP11-829C4	RP11-829C4	RP11-829C4	RP11-829C4	LSIBCR	LSIBCR	LSIBCR	LSIBCR	LSIBCR	LSIBCR	LSIBCR
Deletion size	1.1 Mb	1.1 Mb	1.1 Mb	1.1 Mb	1.1 Mb	1.1 Mb	1.8 Mb	1.8 Mb	1.8 Mb	700 kb	700 kb	700 kb	700 kb
Origin	De novo	Unknown	Not paternal	De novo	Not maternal	De novo	Unknown	Not maternal	De novo	De novo	Unknown	De novo	Unknown
Gender	F	M	M	F	F	M	M	M	F	F	M	M	M
Age (years)	7.8	9	20	10	20	22	2	17	10.5	4.5	3	6	15
Prematurity (GA < 37 weeks)	+	+	NA	+	+	+	+	+	+	+	-	-	-
Delivered by C-section due to severe preeclampsia	Delivered by C-section due to oligohydramnios	Delivered by C-section due to oligohydramnios	Delivered by C-section	Delivered by C-section	Delivered by C-section	Delivered by C-section due to preeclampsia	Delivered by C-section	Spontaneous vaginal delivery	Delivered by C-section	Delivered by C-section	Delivered by C-section	(Full term)	(Full term)
Birth weight	680 g (5th-10th percentile)	1,558 g (<5th percentile)	NA	2,300 g (50th percentile)	1,900 g (10th-50th percentile)	907 g (<5th percentile)	1,470 g (10th-50th percentile)	2,100 g (5th percentile)	782 g (5th-10th percentile)	2,200 g (50th-90th percentile)	3,400 g (10th-90th percentile)	3,600 g (50th-90th percentile)	3,700 g (50th-90th percentile)
Postnatal growth restriction	+	+	+	-	NA	NA	+	-	+	-	-	-	-
Weight at presentation	5th percentile	5th-10th percentile	NA	<5th percentile	NA	NA	10th percentile	NA	<5th percentile	50th percentile	90th percentile	75th-90th percentile	NA
Height at presentation	5th-10th percentile	<5th percentile	<5th percentile	25th percentile	NA	NA	<5th percentile	50th percentile	<5th percentile	25th percentile	90th percentile	90th percentile	NA
Head circumference at presentation	<5th percentile	NA	NA	2nd percentile	NA	NA	<5th percentile	NA	<5th percentile	50th percentile	50th-75th percentile	75th percentile	NA
Developmental delay (DD)	+	+	+	+	+	+	+	+	+	+	+	+	+
Motor and receptive language delays	Global DD	Global DD	Global DD	Global DD	Significant global DD	Global DD, especially language	Global DD, especially language	Language delay with normal motor milestones	Global DD	Global DD	Global DD	Global DD, especially language	Global DD, especially language
Intellectual disability	+	+	+	+	+	??	??	+	+	+	??	+	+
Behavioral problems	Talks to oneself and to imaginary friends; social immaturity	Poor impulse control and anger issues	ADHD; social immaturity; anxiety; impulsivity	ADHD; social immaturity; anxiety; impulsivity	ADHD; Asperger disorder	ADHD	ADHD	Sleep problems; hyperactive behavior	-	-	-	PDD	Autism

ADHD, attention-deficit hyperactivity disorder; CNV, copy-number variation; C-section, cesarean section; DD, developmental delay; F, female; FISH, fluorescence in situ hybridization; GA, gestational age; H/O, history of; M, male; NA, information is not available; PDA, patent ductus arteriosus; PDD, pervasive developmental disorder; ??, patient is too young to assess.

Table 1 Continued on next page

Table 1 Continued

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13
Neurological problems	Poor coordination	H/O focal and generalized seizures	Hypotonia; seizures	–	Seizures	–	–	–	Static encephalopathy	Hypotonia	–	–	Seizures
Cardiovascular defects	–	PDA; ventricular septal defect	–	Benign heart murmur	–	Bicuspid aortic valve	Ventricular septal defect	Atrial septal defect	PDA; atrial and ventricular septal defects	–	–	–	–
Head size/shape and dysmorphic features	Microcephaly; frontal bossing; narrow long face; narrow nose with high nasal bridge; low-set ears; pointed chin	Small triangular face; upturned nares	Prominent forehead; flattened occiput; narrow palpebral fissures	Microcephaly; bitemporal narrowing; synophrys; short upturned nose; full lips; dental crowding	–	NA	Microcephaly; synophrys; arched eyebrows; small upplanting eyes; broad nasal root; small ears; cleft lip/palate	–	Microcephaly; brachycephaly; microphthalmia; deep-set eyes; bilateral cleft lip/palate	Relative macrocephaly; hypertelorism; prominent forehead	Deep-set eyes	–	–
Skeletal anomalies	Bilateral fifth-finger clinodactyly; bilateral valgus deformities	Right claw hand with fused fourth and fifth fingers	Third-finger clinodactyly; marked angulation of left knee; second- and third-toe syndactyly	–	–	Joint contractures	–	Long tapered fingers	–	Bilateral fifth-finger brachydactyly	–	Joint hypermobility	–
Others	Toe walking	Panhypopituitarism; septo-optic dysplasia; small penis	H/O pituitary tumor; small testes and penis	–	–	Ptosis; hypospadias	–	Truncal obesity; delayed puberty; small penis	Retinopathy of prematurity; retinal detachment; small blind in both eyes; left-ear hearing loss and narrow ear canals; G-tube; megacolon	Gastro-esophageal reflux	–	–	–
Other CNVs	–	–	–	–	–	–	–	–	–	–	–	500 kb Duplication at 5q23.3 inherited from clinically normal father	–

ADHD, attention-deficit hyperactivity disorder; CNV, copy-number variation; C-section, cesarean section; DD, developmental delay; F, female; FISH, fluorescence in situ hybridization; GA, gestational age; H/O, history of; M, male; NA, information is not available; PDA, patent ductus arteriosus; PDD, pervasive developmental disorder; ??, patient is too young to assess.

Table 2 Chromosome 22 genomic coordinates of LCR22-A to -H in human genome builds hg18 and hg19

LCR22	NCBI36/hg18	GRCh37/hg19
A	~17,020,000–17,290,000	~18,640,000–18,910,000
B	~18,630,000–19,060,000	~20,250,000–20,680,000
C	~19,350,000–19,420,000	~21,020,000–21,090,000
D	~19,800,000–20,250,000	~21,470,000–21,920,000
E	~21,290,000–21,380,000	~22,960,000–23,050,000
F	~21,980,000–22,150,000	~23,650,000–23,820,000
G	~22,960,000–23,030,000	~24,630,000–24,700,000
H	~23,325,000–23,410,000	~24,995,000–25,080,000

mediating LCR22s, and could be either partially overlapping or nonoverlapping, which suggests that they do not represent a single clinical entity but rather different entities with some difference in their presenting features and risks. In this study, we report 13 new unrelated patients with variable-size deletions in the distal 22q11.2 region as shown by cytogenomic array analyses. We compare our patients' clinical and molecular cytogenetic features with those of previously reported cases in an attempt to better dissect the phenotypic correlations based on the deletion size and position. Finally we propose a system to categorize these deletions based on their genomic position in the distal 22q11.2 region.

MATERIALS AND METHODS

Patients

The 13 patients presented in this report were recruited from the clinical cytogenetics laboratories at the University of Alabama at Birmingham (UAB) Department of Genetics and the Laboratory Corporation of America (LabCorp). All patients have undergone clinical cytogenomic array testing for indications that included DD, ID, autism spectrum disorders, dysmorphic features, multiple congenital anomalies, and general suspicion of chromosomal abnormalities. A total of 28 patients were noted to have a deletion in the distal 22q11.2 region in both laboratories among a total of 35,000 patients referred for clinical cytogenomic array testing (3,000 patients at UAB and 32,000 patients at LabCorp), yielding a frequency of ~0.08%. Seventeen patients had the LCR22-D to -E deletion, three patients had the LCR22-D to -F deletion, two patients had the LCR22-D to -G deletion, and six patients had the LCR22-E to -F deletion. Sufficient clinical information was available for the 13 patients reported here. When parents were available, the de novo or inherited origin of the deletion was investigated using fluorescence in situ hybridization (FISH) analyses. This study was approved by the institutional review board for human subject research at UAB.

G-banded chromosome and FISH analyses

G-banded chromosome and FISH analyses were performed on metaphase preparations of peripheral blood lymphocytes from the patients and their parents using standard techniques. For the

1.1-Mb LCR22-D to -E deletion, the BAC clone RP11-647D11 was used by the cytogenetics laboratory at UAB, whereas the BAC clone RP11-829C4 was used by the cytogenetics laboratory at LabCorp. These clones were prepared from the RPCI-11 human genomic library (Empire Genomics, Buffalo, NY), and the clones' identities were confirmed by FISH analyses on normal metaphase spreads as well as end sequencing. For the 1.8-Mb LCR22-D to -F deletion as well as the small 700-kb LCR22-E to -F deletion, the LSI BCR probe (Abbott, Abbott Park, IL) was used by both labs.

Cytogenomic array analyses

Two cytogenomic array platforms were used. Array comparative genomic hybridization was performed using the 4×44k or the 8×60 k Agilent oligo-arrays (Agilent Technologies, Santa Clara, CA) by the cytogenetics laboratory at UAB. These are custom-designed arrays that are based on the ISCA (International Standards for Cytogenomic Arrays) consortium design. DNA was extracted from the patients' peripheral blood using the Qiagen Blood Mini Kit (Qiagen, Valencia, CA). DNA labeling, slide hybridization, washing, and scanning were performed following the manufacturer's protocol. The arrays were scanned using the Agilent high-resolution microarray scanner (Agilent Technologies). The scanned arrays were analyzed using the Cytogenomics v1.5 software (Agilent Technologies).

SNP microarray analysis was performed by the cytogenetics laboratory at LabCorp, using either the Affymetrix v6.0 or the Affymetrix Cytoscan HD platform (Affymetrix, Santa Clara, CA). DNA labeling, slide hybridization, washing, and scanning were performed following the manufacturer's protocol. The Affymetrix v6.0 chip contains more than 900,000 SNP probes and 900,000 nonpolymorphic copy-number probes with a median spacing of 0.7kb, and the data were analyzed using the Affymetrix Genotyping Console Browser v3.01. The Affymetrix Cytoscan HD contains 743,000 SNP probes and 1,953,000 nonpolymorphic copy-number probes with a median spacing of 0.88kb, and the data were analyzed using Chromosome Analysis Suite (Affymetrix).

RESULTS

Thirteen new unrelated cases were found to have deletions in the distal 22q11.2 region by clinical cytogenomic array testing and were confirmed by metaphase FISH analyses. The patients' clinical and molecular cytogenetic features are summarized in **Table 1**. **Table 2** illustrates the genomic coordinates of LCR22-A to -H in human genome builds hg18 and hg19.

The first significant finding observed is the clear trend for these deletions to be de novo in origin. Parental FISH testing demonstrated that in six patients the deletion was de novo. For three patients, only one parent was available and these were all shown not to have the deletion. In the four remaining patients, the parents were either unavailable or declined parental FISH testing. The second significant observation is the genotypic variability of these deletions. Six patients had the ~1.1 Mb deletion flanked by LCR22-D and -E, three patients had the larger

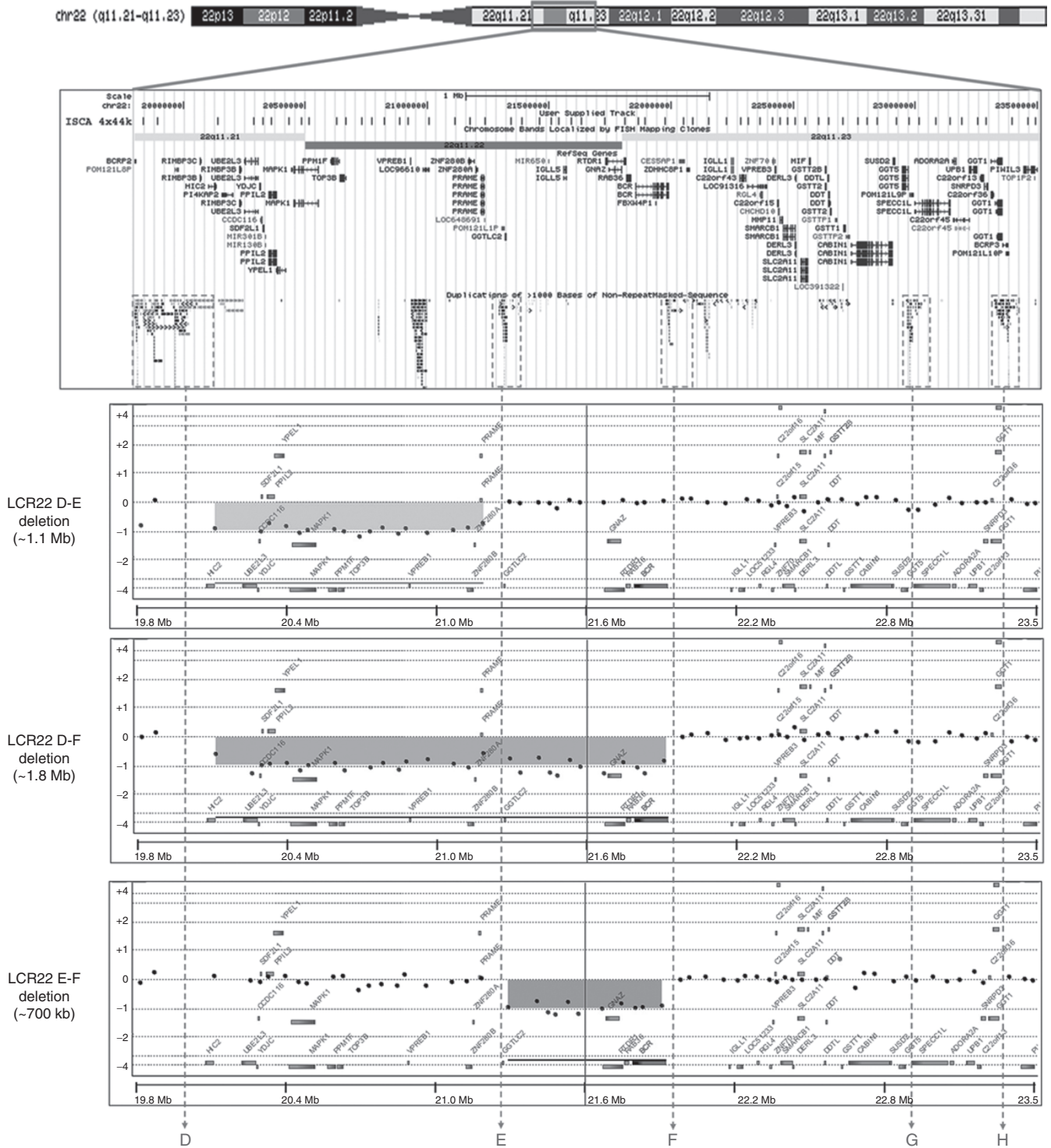


Figure 1 Genomic map of the distal 22q11.2 region (generated using the UCSC genome browser, hg18) aligned with array comparative genomic hybridization plots of overlapping distal 22q11.2 deletions of varying sizes (shaded areas). The dashed boxes and vertical lines represent the LCR22-D to -H.

~1.8 Mb deletion flanked by LCR22-D and -F, and four patients had the small ~700 kb deletion flanked by LCR22-E and -F (Figures 1 and 2). No other clinically significant CNVs were noted in any patient, with the exception of patient 12, who also carried a 500 kb duplication at 5q23.3 inherited from his

clinically normal father; this was thought to be a benign variant—arr[hg19] 5q23.3(127,751,662–128,250,700)x3 pat.

All patients were found to share many of the clinical features previously reported to be characteristic of the distal 22q11.2 microdeletion syndrome, including global DD, ID, and mild

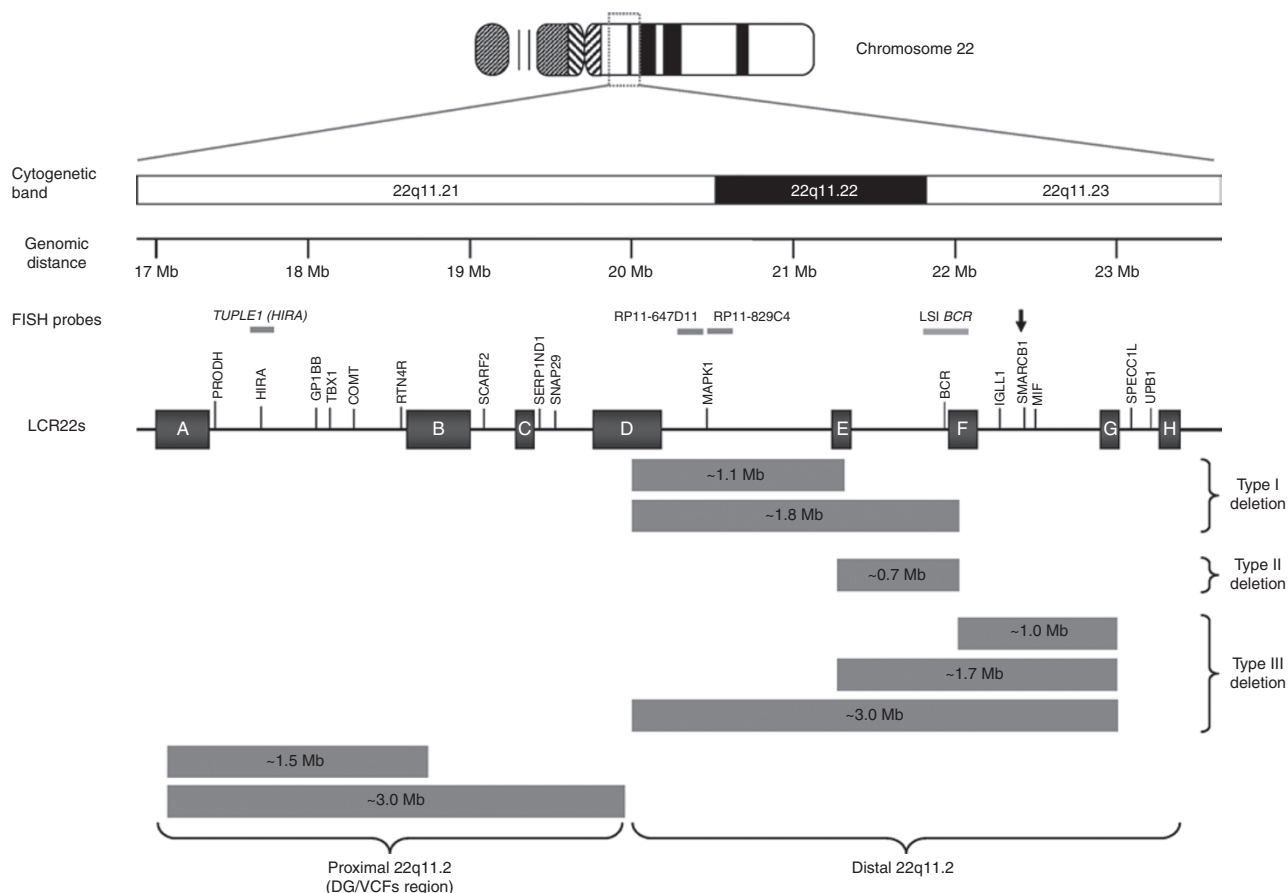


Figure 2 Schematic representation of the 22q11.2 region and the recurrent microdeletions reported in this region. Cytogetic bands and genomic ruler are shown (hg18). FISH probes used are illustrated by the horizontal bars. The eight LCR22 clusters are illustrated by the green boxes and labeled A to H. The red horizontal bars below the map depict the reported deletions at both the proximal and distal 22q11.2 regions (refs. 7–18). The proximal 22q11.2 region corresponds to the DG/VCFs typically deleted region. The distal 22q11.2 microdeletions can be categorized into three types: type I: with deletions flanked by LCR22-D and either -E or -F, type II: with deletions flanked by LCR22-E and -F, and type III: with any deletion in this region minimally spanning the LCR22-F to -G interval and encompassing the *SMARCB1* gene (black arrow). DG/VCFs, DiGeorge/velocardiofacial syndrome; FISH, fluorescence in situ hybridization.

dysmorphic features. However, there was a noticeable degree of phenotypic variability that could be partially explained by the genotypic variability. For all patients for whom we have gestational age data (12 of 13 patients), 9 patients were born prematurely with a gestational age ranging between 27 and 36 weeks. Those born prematurely were mostly delivered by C-section due to causes that included preeclampsia and oligohydramnios. Preterm birth was noted in eight of eight patients (100%) with either the LCR22-D to -E deletion or the LCR22-D to -F deletion, whereas it was noted in one of four patients (25%) with the small LCR22-E to -F deletion.

Prenatal growth restriction as evidenced by a low birth weight for gestational age (<10th percentile) was noted in five patients, whereas seven patients had a birth weight between the 10th and 90th percentile. The birth weight was not available for patient 3. Five patients had a history of postnatal growth restriction, with height consistently below the 10th percentile, whereas six patients had postnatal growth that was within the normal range. In the two remaining patients, postnatal weight and height data were not available. Collectively, pre- and/or

postnatal growth restriction was noted in seven of nine patients (78%) with either the LCR22-D to -E deletion or the LCR22-D to -F deletion, whereas no growth restriction was noted in the four patients with the small LCR22-E to -F deletion.

Global DD and mild to moderate ID were universal clinical features in all patients. The DD was noticeably more prominent in language (Table 1). Eight patients suffered from various behavioral problems, which included social immaturity, talking to oneself, poor impulse control, anger issues, sleep problems, attention-deficit hyperactivity disorder, and autism spectrum disorders. Seven patients showed evidence of neurological problems, which included poor coordination, hypotonia, and seizures, with the most severely affected patient presenting with static encephalopathy. The behavioral and neurological problems were noted in all deletion types (Table 1).

Cardiovascular defects were noted in five of nine patients (56%) with either the LCR22-D to -E deletion or the LCR22-D to -F deletion, whereas no cardiovascular defects were noted in the four patients with the small LCR22-E to -F deletion. The cardiovascular defects observed in our patients varied in

severity and included patent ductus arteriosus, atrial and ventricular septal defects, and bicuspid aortic valve (Table 1).

Physical examination revealed nonspecific facial dysmorphic features in eight patients (Table 1). These included narrow long face or small triangular face, prominent forehead, bitemporal narrowing, flattened occiput, arched eyebrows, synophrys, narrow palpebral fissures, small upslanting eyes, deep-set eyes, low-set small ears, broad nasal root, short upturned nose, and pointed chin. Two patients had cleft lip and palate.

Minor skeletal abnormalities were noted in seven patients, including bilateral fifth-finger clinodactyly and valgus deformity in patient 1, right claw hand with fused fourth and fifth fingers in patient 2, third-finger clinodactyly with marked angulation of the left knee and second- and third-toe syndactyly in patient 3, joint contractures in patient 6, long tapered fingers in patient 8, bilateral fifth-finger brachydactyly in patient 10, and joint hypermobility in patient 12. Other clinical problems noted in our patients included panhypopituitarism with septo-optic dysplasia in patient 2, history of pituitary tumor in patient 3, ptosis in patient 6, and truncal obesity with gynecomastia in patient 8. Three patients (patients 2, 3, and 8) had small testes and/or small penis, with one of them also presenting with delayed puberty. Patient 9 also presented with retinopathy of prematurity, which progressed to retinal detachment and eventually she became blind in both eyes. She also suffered from left-ear hearing loss with narrow ear canals, as well as megacolon; she was G-tube-dependent for feeding.

DISCUSSION

The previously purported distal 22q11.2 microdeletion syndrome (OMIM 611867) in the region immediately distal to the DG/VCFs recurrent microdeletion region has been reported to be associated with a variable phenotype that includes core features in the form of preterm birth, pre- and/or postnatal growth restriction, DD, ID, behavioral problems, cardiovascular defects, skeletal abnormalities, and mild dysmorphic facial features.⁷⁻¹⁸ These deletions are mediated by nonallelic homologous recombination between the five telomeric LCR22s (LCR22-D to -H) in the distal portion of the 22q11.2 region (Figure 2).¹⁰ Distal 22q11.2 microdeletions that span the LCR22-F to -G interval encompassing the tumor suppressor *SMARCB1* gene (also called *INI1*) have been reported to manifest many of the presenting features mentioned above but also have high incidence of malignant rhabdoid tumors in infancy and early childhood, predominantly in the kidneys and central nervous system, which necessitates tumor surveillance in these patients.¹⁹⁻²⁴

Depending on the mediating LCR22s, the distal 22q11.2 microdeletions can vary in size between ~700 kb and ~3.0 Mb. To date, the distal 22q11.2 microdeletions have been grouped together as a single clinical entity despite the fact that these deletions comprise several partially overlapping and nonoverlapping deletions of varying size. In this study we present 13 new unrelated patients with variable-sized deletions in the distal 22q11.2 region. A near-universal feature noted in all except

three of our patients (patients 11, 12, and 13) is preterm birth with a gestational age that varied between 27 and 36 weeks. Patients 11, 12, and 13 were born at full term and carried the small LCR22-E to -F deletion. Taken together, our results strengthen the association between the distal 22q11.2 deletions that span the LCR22-D to -E interval and preterm birth but suggest that preterm birth is not a common feature in patients with the small LCR22-E to -F deletion. Preterm birth is not a common feature of chromosomal aberrations in general, and has not been described in the DG/VCFs. It is worth noting that some of the severe presenting features observed in our patients can be directly attributed to very early preterm birth, as in the case of patient 9, who suffered from static encephalopathy and retinopathy of prematurity that was complicated by retinal detachment and blindness.

Approximately 78% (7 of 9) of the patients in this study with the 1.1-Mb LCR22-D to -E deletion and the larger 1.8-Mb LCR22-D to -F deletion, and whose birth weight and postnatal weight/height data were available, showed evidence of pre- and/or postnatal growth restriction. The four patients with the small 700-kb LCR22-E to -F deletion had normal pre- and/or postnatal growth curves, which suggest that the critical region for growth restriction maps to the LCR-D to -E interval. This region encompasses 22 annotated *RefSeq* genes, including the *MAPK1* gene. *MAPK1* encodes mitogen-activated protein kinase 1 and has been proposed to be a candidate for the prematurity and/or low birth weight in distal 22q11.2 microdeletions due to the fact that it is involved in placental development.¹² These data suggest that pre- and postnatal growth restriction with short stature are common features of the distal 22q11.2 deletions that span the LCR22-D to -E interval but not of those that span the LCR22-E to -F interval.

Global DD and ID with a significant language delay component were noted in all our patients, including those with the small 700-kb LCR22-E to -F deletion. DD and/or ID have been reported in more than 80% of distal 22q11.2 microdeletion patients described in the literature to date and tend to be relatively mild to moderate in severity.⁷⁻¹⁸ Taken together, global DD and ID seem to be key components of the distal 22q11.2 microdeletions phenotype irrespective of the size and position of the deletion. Behavioral and neurological problems were also quite common in our patients in all deletion types. The behavioral problems included social immaturity, poor impulse control, anger issues, sleep problems, attention-deficit hyperactivity disorder, and autism spectrum disorders, whereas the neurological problems included poor coordination, hypotonia, and seizures, with the most severely affected patient presenting with static encephalopathy.

Congenital cardiovascular defects were observed in ~50% of distal 22q11.2 microdeletion patients and include septal defects, truncus arteriosus, bicuspid aortic valve, and cardiac dextrorotation.^{8,9,12,15,17,18} A similar frequency of cardiac defects was noted in our study, with five patients (~42%) presenting with a history of a cardiac defect, including patent ductus arteriosus, atrial and ventricular septal defects, and bicuspid aortic

valve. It is worth noting that the four patients with the small 700-kb LCR22-E to -F deletion did not have history of a cardiac defect, again suggesting that the critical region for cardiac defects maps to the LCR-D to -E interval.

Only one patient with the small LCR22-E to -F deletion was previously reported in the literature.⁹ This patient had a congenital heart defect with normal growth, appearance, and psychomotor development. On careful examination, she was found to have slightly broad folding of the right helix and widely spaced inverted nipples. Her father carried the same deletion and had narrow palpebral fissures and low-set ears but no heart defects. Seven more patients were reported in the DECIPHER Database with this small LCR22-E to -F deletion.²⁵ The clinical features of five of them (patients 1579, 255108, 258704, 265081, and 271344) were available, and included mostly DD, ID, and mild dysmorphic features. No preterm birth, growth restriction, or heart defects were reported. This suggests that this small LCR22-E to -F deletion is relatively rare and/or has a mild phenotype. However, ascertainment bias due to the milder phenotype cannot be excluded, which might be a confounder in establishing the phenotypic correlations and relative frequency of this small deletion.

Dysmorphic facial features commonly reported in distal 22q11.2 microdeletion patients include arched eyebrows, deep-set eyes, smooth philtrum, thin upper lip, hypoplastic alae nasi, and small pointed chin.^{7–18} Moreover, some patients present with cleft lip and/or cleft palate. Although some of our patients manifested some of these features, the majority of them presented with nonspecific, nondiagnostic features that were quite variable, as shown in the Results section.

In conclusion, we suggest that the recurrent distal 22q11.2 microdeletions do not represent a single clinical entity but vary depending on the specific mediating LCR22s and the intervening gene content. Our data suggest that the 1.1-Mb LCR22-D to -E deletion and the 1.8-Mb LCR22-D to -F deletion have common presenting features similar to the ones reported in the literature. The small 700-kb LCR22-E to -F deletion, however, seems to be relatively less frequent and to have a milder phenotype that is less likely to include preterm birth, pre- and postnatal growth restriction, and cardiovascular defects. Patients who carry this small deletion share with the larger 1.1-Mb and 1.8-Mb deletion patients the other common presenting features, including the DD, ID, and minor nondiagnostic dysmorphic features. This observation, however, waits to be confirmed in other studies due to the relatively small number of patients with the small 700-kb LCR22-E to -F deletion presented here and in the literature. On the other hand, deletions in the distal 22q11.2 region that span the LCR22-F to -G interval including the *SMARCB1* gene have been reported in multiple studies and were shown to be also variable in size and share many of the presenting features of the distal 22q11.2 microdeletion phenotype discussed earlier, in addition to a very high incidence of malignant rhabdoid tumors in infancy and early childhood.^{19–24}

Accordingly, we propose categorizing the distal 22q11.2 microdeletions into three genomic types: type I: with deletions

flanked by LCR22-D and either -E or -F; type II: with deletions flanked by LCR22-E and -F; and type III: with any deletion in this region minimally spanning the LCR22-F to -G interval and encompassing the *SMARCB1* gene (Figure 2). All three deletion types are thought to be pathogenic and are most often de novo. They all share many of the presenting features mentioned above but also have their unique features and risks. Type II deletion seems to be relatively less frequent and to have a milder phenotype that is less likely to include preterm birth, pre- and postnatal growth restriction, and cardiovascular defects, whereas type III deletions are characterized by a very high incidence of malignant rhabdoid tumors in infancy and early childhood. This underscores the need for accurate breakpoint mapping and sizing of these deletions in clinical practice.

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DISCLOSURE

The authors declared no conflict of interest.

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