Central 22q11.2 Deletions

Rump, Patrick; de Leeuw, Nicole; van Essen, Anthonie J.; Verschuuren - Bemelmans, Corien C.; Veenstra-Knol, Hermine E.; Swinkels, Marielle E. M.; Oostdijk, Wilma; Ruivenkamp, Claudia; Reardon, Willie; de Munnik, Sonja

Published in:
American Journal of Medical Genetics. Part B: Neuropsychiatric Genetics

DOI:
10.1002/ajmg.a.36711

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Central 22q11.2 Deletions

Patrick Rump,[1]* Nicole de Leeuw,[2] Anthonie J. van Essen,[1] Corien C. Verschuuren-Bemelmans,[1] Hermine E. Veenstra-Knol,[1] Mariëlle E.M. Swinkels,[3] Wilma Oostdijk,[4] Claudia Ruivenkamp,[5] Willie Reardon,[6] Sonja de Munnik,[2] Mariken Ruiter,[2] Ayala Frumkin,[7] Dorit Lev,[8] Christina Evers,[9] Birgit Sikkema-Raddatz,[1] Trijnie Dijkhuizen,[1] and Conny M. van Ravenswaaij-Arts[1]

[1]University of Groningen, University Medical Centre Groningen, Department of Genetics, Groningen, The Netherlands

[2]Department of Human Genetics, Radboud University Medical Centre, Nijmegen, The Netherlands

[3]Department of Medical Genetics, University Medical Centre Utrecht, Utrecht, The Netherlands

[4]Department of Pediatrics, Leiden University Medical Centre, Leiden, The Netherlands

[5]Department of Genetics, Leiden University Medical Centre, Leiden, The Netherlands

[6]National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Crumlin, Dublin, Ireland

[7]The Monique and Jacques Roboh Department of Genetic Research, Hadassah, Hebrew University Medical Centre, Jerusalem, Israel

[8]Institute of Medical Genetics, Wolfson Medical Centre, Holon, Israel

[9]Institute of Human Genetics, Heidelberg University, Heidelberg, Germany

Manuscript Received: 5 March 2014; Manuscript Accepted: 29 May 2014

22q11.2 deletion syndrome is one of the most common microdeletion syndromes. Most patients have a deletion resulting from a recombination of low copy repeat blocks LCR22-A and LCR22-D. Loss of the *TBX1* gene is considered the most important cause of the phenotype. A limited number of patients with smaller, overlapping deletions distal to the *TBX*1 locus have been described in the literature. In these patients, the *CRKL* gene is deleted. Haploinsufficiency of this gene has also been implicated in the pathogenesis of 22q11.2 deletion syndrome. To distinguish these deletions (comprising the LCR22-B to LCR22-D region) from the more distal 22q11.2 deletions (located beyond LCR22-D), we propose the term "central 22q11.2 deletions". In the present study we report on 27 new patients with such a deletion. Together with information on previously published cases, we review the clinical findings of 52 patients. The prevalence of congenital heart anomalies and the frequency of de novo deletions in patients with a central deletion are substantially lower than in patients with a common or distal 22q11.2 deletion. Renal and urinary tract malformations, developmental delays, cognitive impairments and behavioral problems seem to be equally frequent as in patients with a common deletion. None of the patients had a cleft palate. Patients with a deletion that also encompassed the *MAPK1* gene, located just distal to LCR22-D, have a different and more severe phenotype, characterized by a higher prevalence of congenital heart anomalies, growth restriction and microcephaly. Our results further elucidate genotype-phenotype correlations in 22q11.2 deletion syndrome spectrum.

Key words: 22q11.2; deletion; atypical; distal; *TBX1*; *CRKL*; *MAPK1*

How to Cite this Article:
Rump P, de Leeuw N, van Essen AJ, Verschuuren-Bemelmans CC, Veenstra-Knol HE, Swinkels MEM, Oostdijk W, Ruivenkamp C, Reardon W, de Munnik S, Ruiter M, Frumkin A, Lev D, Evers C, Sikkema-Raddatz B, Dijkhuizen T, van Ravenswaaij-Arts CM. 2014. Central 22q11.2 deletions.
Am J Med Genet Part A 164A:2707–2723.

INTRODUCTION

Chromosomal microdeletions are an important cause of intellectual disability and congenital anomalies. With an estimated occurrence in approximately 1:4000 live births, 22q11.2 deletion syndrome is one of the most common microdeletion syndromes [Lindsay, 2001; Óskarsdóttir et al., 2004; McDonald-McGinn and Sullivan, 2011]. The region involved on the long arm of chromosome 22 contains a high number of low copy repeat sequences

Conflict of Interest: The authors declare no conflict of interest.
*Correspondence to:
Patrick Rump, M.D., Ph.D., Department of Genetics, University Medical Centre Groningen, P.O. Box 30.001, 9700 RB Groningen, the Netherlands.
E-mail: p.rump@umcg.nl
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 14 August 2014
DOI 10.1002/ajmg.a.36711

(LCRs) (Fig. 1). Non-allelic homologous recombinations between these LCRs cause a spectrum of recurrent rearrangements, including deletions and duplications [Edelmann et al., 1999; Saitta et al., 2004; Shaikh et al., 2007]. In total, the region contains eight LCRs blocks, known as LCR22-A to LCR22-H. The most common deletion occurs between the two largest blocks, LCR22-A and LCR22-D, and results in a ~3 Mb deletion [Edelmann et al., 1999; Emanuel, 2008]. This deletion has been associated with highly variable phenotypes: velo-cardio-facial syndrome [OMIM 192430], DiGeorge syndrome [OMIM 188400], Cayler cardiofacial syndrome [OMIM 125520], and conotruncal anomaly face or Takao syndrome [OMIM 217095]. All these presentations of the same disorder are now collectively referred to as 22q11.2 deletion syndrome. The wide range of clinical findings in 22q11.2 deletion syndrome include cardiovascular malformations, palatal abnormalities, urogenital anomalies, craniofacial features, developmental and learning problems, intellectual disability, behavioral and psychiatric problems, immune deficiencies, and congenital hypocalcemia [Ryan et al., 1997; Lindsay, 2001; McDonald-McGinn and Sullivan, 2011]. The second most common deletion is a nested ~1.5 Mb proximal 22q11.2 deletion, caused by recombination between LCR22-A and LCR22-B. The phenotype associated with this proximal deletion seems to be indistinguishable from that seen in patients with the larger ~3 Mb deletion [Carlson et al., 1997; Bartsch et al., 2003]. Haploinsufficiency of the *TBX1* gene [OMIM 602054] is thought to be the most important cause of the clinical features seen in association with these two deletions [Lindsay, 2001; McDonald-McGinn and Sullivan, 2011].

More recently, a recurrent but distinct microdeletion syndrome was described that is caused by deletions distal from the ~3 Mb common deletion [Rauch et al., 1999; Mikhail et al., 2007; Rodningen et al., 2008; Ben-Shachar et al., 2008; Fagerberg et al., 2013]. These distal 22q11.2 deletions occur mostly between LCR22-D and either LCR22-E, LCR22-F or LCR22-G [Ben-Shachar et al., 2008]. Characteristic features associated with this distal 22q11.2 deletion syndrome are prematurity, prenatal and postnatal growth restriction, learning problems, developmental delay, specific facial characteristics, mild skeletal abnormalities, and an increased incidence of a specific type of heart defect, truncus arteriosus. It has been suggested that haploinsufficiency of the *MAPK1* gene [OMIM 176948] might be responsible for the growth restriction and several of the observed malformations, including the congenital heart anomalies [Ben-Shachar et al., 2008; Tan et al., 2011; Fagerberg et al., 2013].

Deletions of the region located between LCR22-B and LCR22-D (distal from the ~1.5 Mb proximal deletion and proximal to the above mentioned distal 22q11.2 deletions) seem to be relatively rare. Only a limited number of patients with such deletions have been described in the literature, usually denoted as distal or atypical 22q11.2 deletions [Kurahashi et al., 1997; Garcia-Minaur et al., 2002; Rauch et al., 2005; D'Angelo et al., 2007; Fernandez et al., 2009; Ogilvie et al., 2009; Garavelli et al., 2011; Ledig et al., 2011; Yu et al., 2011; Breckpot et al., 2012; Sanna-Cherchi et al., 2012; Verhagen et al., 2012]. To avoid confusion with 22q11.2 deletions distal from the common deleted region, we suggest calling these "central 22q11.2 deletions." Studies in mice have indicated that loss of the *CRKL* gene [OMIM 602007], that resides in central 22q11.2 region can cause several phenotypic features of the 22q11.2 deletion syndrome seen in humans [Guris et al., 2001; Moon et al., 2006]. Here we report on the clinical findings for 27 new patients from 15 unrelated families, in whom we found a central 22q11.2 deletion with loss of *CRKL*, but without loss of *TBX1*. Combining these new patients with previously reported patients, we then reviewed the clinical findings for 52 subjects with a central 22q11.2 deletion. This gave us an opportunity to investigate whether there are new breakpoint-specific clinical phenotypes in the widening spectrum of 22q11.2 deletion syndromes.

MATERIALS AND METHODS

We included 27 patients from 15 unrelated families with a submicroscopic deletion involving the LCR22-B to LCR22-D region at chromosome 22q11.2 in this collaborative study. Clinical information was retrieved from medical records or provided by the referring physicians. Genomic DNA was isolated according to standard procedures. Because all deletions were found during the diagnostic work-up of the probands, different microarray platforms were used depending on when and where the work-up took place. Array analysis was performed in families 1–5, 7, 13, and 15 by oligonucleotide arrays (Agilent Technologies, Santa Clara, CA, USA) and in families 6, 8–12, and 14 by single nucleotide polymorphism (SNP) arrays (Affymetrix Inc., Santa Clara, CA, USA) (Table I). Copy-number variations were determined using DNA analytics software (versions 4.0.73 or 4.0.81), Copy Number Analyzer for Affymetrics GeneChip mapping software (version 2.0) or Affymetrix Chromosome Analysis Suite software, depending on the platform that was used. The normalized ratios were analyzed for losses and gains of chromosome regions using previously described methods [de Vries et al., 2005; Hehir-Kwa et al., 2007]. All results were evaluated using the UCSC human Genome Browser release of February 2009 (GRCh37/hg19). When parents or other family members were available for testing, de novo or inherited occurrence of the deletions was determined.

CLINICAL REPORTS

Family 1

The proband (Patient 1) was the first child of nonconsanguineous parents. A unilateral renal agenesis with an absent ipsilateral renal artery was detected prenatally. He was born after 39^{+5} weeks gestation with a birth weight of 3,180 grams (−0.2 SD). He received prophylactic treatment with antibiotics during the first postnatal month. There were no additional complications during the neonatal period and infancy. Between the age of one and two years, he had a few middle ear infections. At the age of three years, a mild delay of speech development was noted for which he received speech therapy. He was otherwise healthy. His appearance was characterized by a triangular, asymmetric face with a broad forehead, high nasal bridge, deep-set eyes, and mildly upslanting palpebral fissures. His mother had two subsequent first-trimester spontaneous abortions. Standard chromosome analyses in both parents showed normal karyotypes. During her fourth pregnancy, a normal sized male fetus (Patient 2) in breech position with oligohydramnion was seen on ultrasound, and bilateral enlarged cystic kidneys were

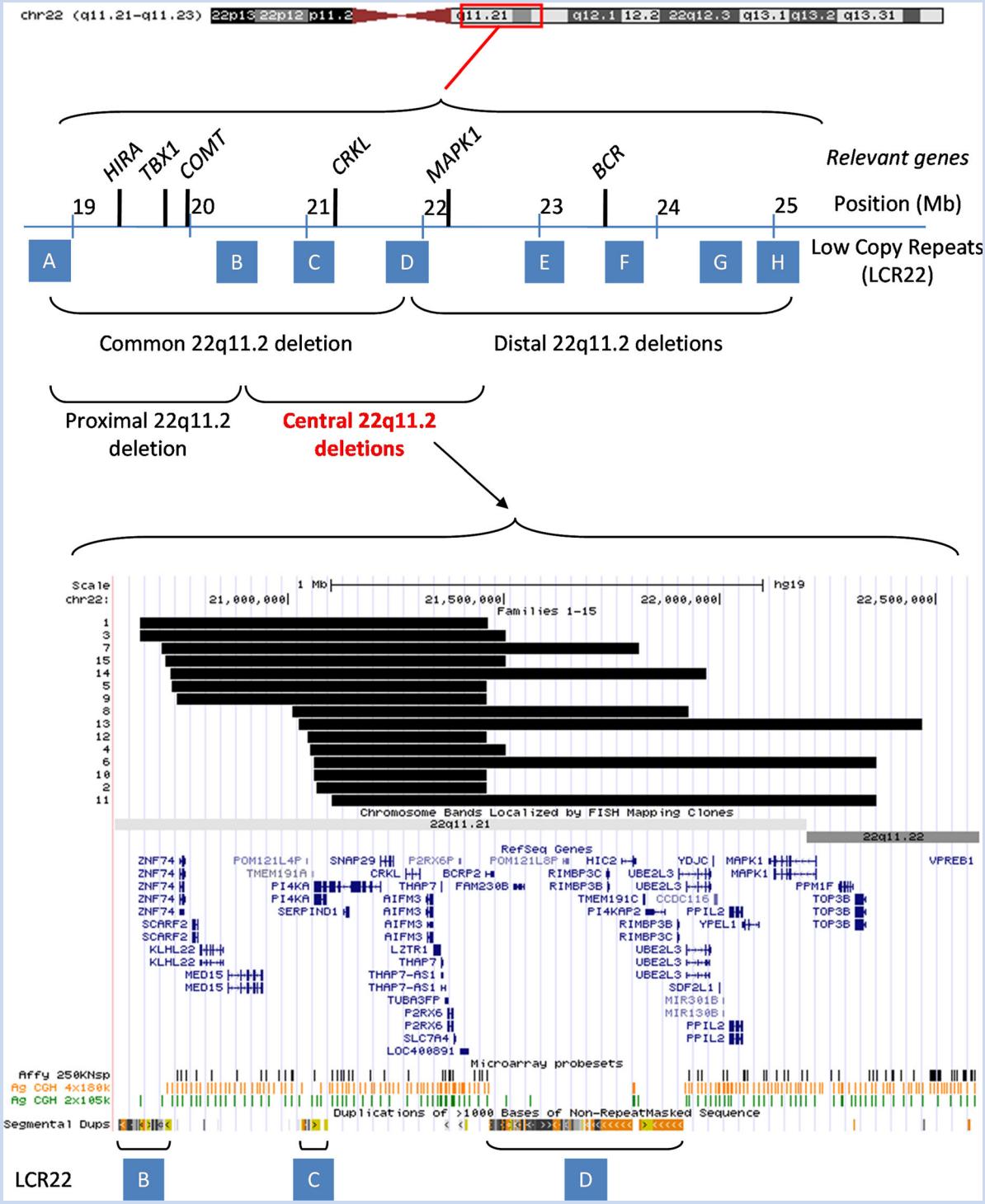

FIG. 1. Schematic representation of chromosome 22q11.2. The positions of genes and deletions are shown relative to the low copy repeat blocks within the region (LCR22-A to LCR22-H). The magnified image of the central chromosome 22q11.2 region was made using UCSC Genome Browser Build 37/hg19 (http://genome.ucsc.edu/). Black bars depict the 15 central 22q11.2 deletions described in this study, which are ordered by their proximal breakpoints. The Ref Seq gene content is shown, as well as the probe distributions of the three most common array platforms used in this study. [Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833]

TABLE I. Results of Microarray Analysis in 15 Families with Central 22q11.2 Deletions

Family	Size (kb)	Position [a]	Platform	Deletion type	Inheritance
1	805	20,659,346-21,464,259	Agilent #15/#22 ID019630	B to D	paternal
2	396	21,065,681-21,461,951	Agilent 105K ID019015	C to D	maternal
3	846	20,659,346-21,505,557	Agilent #15/#22 ID019630	B to D	unknown
4	452	21,053,694-21,505,558	Agilent 180K ID23363	C to D	paternal
5	729	20,733,226-21,461,951	Agilent 105K ID019015	B to D	paternal
6	1300	21,060,359-22,360,650	Affymetrix 250K Nsp SNP	C to beyond D	de novo
7	1103	20,708,690-21,812,179	Agilent 180K ID23363	B to D	de novo
8	918	21,011,216-21,928,916	Affymetrix 250K Nsp SNP	C to D	unknown
9	719	20,742,450-21,461,607	Affymetrix 250K Nsp SNP	B to D	de novo
10	401	21,060,359-21,461,607	Affymetrix 250K Nsp SNP	C to D	maternal
11	1257	21,103,551-22,360,650	Affymetrix 250K Nsp SNP	C to beyond D	de novo
12	414	21,046,144-21,460,009	Affymetrix 2.7M Cytogenetics	C to D	maternal
13	1442	21,025,454-22,467,502	Agilent 180K ID23363	C to beyond D	de novo
14	1239	20,727,938-21,967,247	Affymetrix 2.7M Cytogenetics	B to D	unknown
15	786	20,718,912-21,505,558	Agilent 180K ID23363	B to D	paternal

[a]NCBI human genome build Hg 19 (http://genome.ucsc.edu/).

noted. Besides a small thorax suspected for lung hypoplasia, no other anomalies were evident. Amniocentesis showed a normal male karyotype (46,XY). The pregnancy was terminated at approximately 23 weeks gestation. This second child had a mild micrognathia, low-set ears with overfolded helices, and flexion and endorotation contractures of both feet. At autopsy, a dysplastic horseshoe kidney with multiple, variably sized cysts, thin underdeveloped ureters and an abnormal bladder with a septum were seen. An ultrasound investigation revealed no renal or urological anomalies in the mother. The father (Patient 3), however, was born with a small dysplastic non-functional right kidney, which was removed at the age of five years. He had a right-sided ectopic ureter orifice that ended within the prostatic urethra. The distal part of the ureter was wide and there was ipsilateral vesicoureteral reflux. A bladder diverticulum was also present. The father had dyslexia and had attended a special primary school for children with learning problems, but he was able to complete a normal secondary school education. He had a long asymmetric face, broad forehead, deep-set eyes, mildly up-slanting palpebral fissures, prominent chin and a mild thoracic scoliosis. A cardiologic and ophthalmologic evaluation showed no abnormalities. Microarray analysis in the proband showed an 805 kb deletion of chromosome 22q11.2. The deletion was also found in the affected fetus and the father. The deletion was not detected in the mother, and was absent in the paternal grandparents.

Family 2

The proband (Patient 4) was born with a right-sided unilateral renal agenesis and a normal left kidney after an uncomplicated pregnancy and delivery. He also had a left-sided inguinal hernia, with an ipsilateral cryptorchid testis, absent epididymis, and ductus deferens. At seven years of age, his height was 128.5 cm (−1 SD), weight 25 kg (0 SD), and head circumference 53 cm (−0.5 SD). He had a thin vermilion of the upper lip and an overfolded helix of the left ear. A cardiologic evaluation showed no abnormalities. He had normal motor development and normal intelligence. His mother (Patient 5) had a partial atrio-ventricular septal defect and mitral valve incompetence. During her teens, she developed a mild spastic paraplegia with an abnormal gait, clumsiness, tremor, muscle cramps, and pain. She had a borderline intelligence, attended a special primary school, and did not complete her secondary school education. Upon evaluation at age 30 years, the mother had a square face with up-slanting palpebral fissures and slightly overfolded helices of the ears. She had relative microcephaly. Her height was 182 cm (+1.75 SD) and her head circumference was 54.5 cm (−0.5 SD). She also had a thoracic kyphosis, increased tendon reflexes, Babinski reflexes, bilateral pes cavus, and hammer toes. An EMG showed no evident signs of a polyneuropathy. MRI scans of her brain and spinal cord revealed no explanatory abnormalities either. Spondylolisthesis of L5-S1, height reduction of T7, and irregular end plates of T7-8 were seen on a spine radiograph. Her karyotype was 47,XXX. Microarray analysis showed a 396 kb 22q11.2 deletion in both the proband and his mother. The maternal grandfather also had pes cavus, but he was not available for genetic testing.

Family 3

The proband (Patient 6) was first seen by a pediatrician at the age of four years because of mild cognitive impairment and behavioral problems. He was born at term after an uncomplicated pregnancy and delivery. His birth weight was 3,400 grams (0 SD). His motor development and speech development were both delayed. He had frequent and severe middle ear infections, which resulted in a conductive hearing loss. At age four years, he avoided eye contact and sometimes showed aggressive behavior towards other children. Both an attention deficit and hyperactivity disorder (ADHD) and a

pervasive developmental disorder - not otherwise specified (PDD-NOS) were diagnosed. His total IQ score was 78. He attended special primary and secondary schools for children with learning and behavioral problems. Upon physical examination, his height was 110 cm (0 SD), his weight was 25.8 kg (>2 SD), and his head circumference was 50.6 cm (−0.5 SD). He had a mildly triangular, asymmetric face with a broad forehead and an open mouth with a full lower lip. His palate and uvula were normal. Apart from overriding toes on both feet, no other anomalies were seen. A renal ultrasound and an ophthalmologic and cardiologic evaluation showed no abnormalities. Fragile-X syndrome was excluded by DNA analysis and he had a normal male karyotype (46,XY). Microarray analysis showed an 846 kb 22q11.2 deletion, which was not detected in his father. Both his father and a younger paternal half-brother had attention deficit disorder (ADD). The mother of the proband was not available for genetic testing.

Family 4

The proband (Patient 7) was born at 36 weeks gestation after an uncomplicated pregnancy. His birth weight was 3,700 g. He was referred for a genetic evaluation at the age of eight years because of a mild intellectual disability and an anxiety disorder. His total IQ score was 76. He had ADD, dyslexia, aggressive behavioral outbursts, recurrent middle ear infections, and clumsy motor skills. Height, weight, and head circumference were all normal. His face was hypotonic, with an open-mouthed appearance and nasal speech. His palate was high and narrow and he had a severe overbite. He also had epicanthic folds, peri-orbital fullness, mild ptosis of the left eye, an upturned nose, increased tendon reflexes, and an intention tremor. A brain MRI showed no abnormalities. Ultrasound investigations showed a mild hydronephrosis of the left kidney but no structural heart anomalies. Fragile-X syndrome was excluded by DNA analysis and he had a normal male karyotype (46, XY). However, microarray analysis showed a 452 kb 22q11.2 deletion. The deletion was also detected in his father (Patient 8), two brothers (Patients 9 and 10), and paternal grandfather (Patient 11). The father had similar facial features to the proband and also had clumsy motor skills, a mild tremor, and learning problems during childhood. A renal ultrasound showed a mild hydronephrosis. One of the brothers (Patient 9) was treated for phymosis, pyelonephritis, and an uretero-pelvic junction stenosis. This brother also had a delay in speech and motor development. The other brother (Patient 10) had neonatal hypotonia and feeding problems, a developmental and growth delay, clumsy motor skills, dyslexia, and PDD-NOS. He had a triangular face, slender build, and long fingers. His renal and cardiac ultrasounds were normal. The grandfather (Patient 11) had a small diaphragmatic hernia, mild tremor, long face with short palpebral fissures, and a high narrow palate. A cardiologic evaluation showed ventricular extra systoles and dilated atria, but no congenital anomalies. He also had a single renal cyst.

Family 5

The 16-year-old male proband (Patient 12) was born with a perimembranous ventricular septal defect and a right-sided inguinal hernia. A diastasis recti was noted during childhood and he suffered from frequent middle ear infections. From the age of seven years onward, he had repeated periods of steroid-dependent proteinuria. The findings of a renal biopsy were consistent with an IgM nephropathy. The meatus of his urethra was stenotic. He had ADD that did not respond to treatment with methylphenidate. His intelligence was above average, and he attended a normal secondary school. On physical examination, his height was 169.5 cm (−1.5 SD), his weight was 54 kg (0 SD) and his head circumference was 57.5 cm (+0.5 SD). He had a long face with narrow palpebral fissures and a flat malar region. Pits were seen at the back of both earlobes. His nostrils were narrow and the nasal cartilage seemed absent. He had a high palate and the second upper left molar was missing. The ability to open his mouth was limited, which was caused by symmetric bony outgrowths on both zygomatic arcs that were visible on a CT scan. In addition, he had a pectus excavatum, long hands, and a mild bilateral 2–3 syndactyly of the toes. He had a normal male karyotype (46,XY). Microarray analysis showed a 729 kb 22q11.2 deletion, which was also detected in his asymptomatic father (Patient 13).

Family 6: A heart malformation was suspected prenatally in the female proband (Patient 14). Amniocentesis showed a normal female karyotype (46,XX). She was born at 35^{+6} weeks gestation with a birth weight of 2,030 g (−0.5 SD). Her length at birth was 43 cm (−1 SD) and her head circumference 29.3 cm (−2 SD). She had a ventricular septal defect with an overriding aorta. In addition, she had a hydrosyringomyelia. Her face was flat with a flat nose, hypoplastic alae nasi, downturned corners of the mouth, thin vermilion of the upper lip and square shaped ears with uplifted earlobes and overfolded helices. Her fingers were slightly tapering and there was a mild clinodactyly of the fifth fingers. A brain MRI was normal and no abnormalities were seen on a renal ultrasound. At 15 months of age, a mild immunodeficiency was found with slightly reduced levels of T-helper lymphocytes and increased levels of natural killer cells. She had a postnatal growth restriction, with height, weight and head circumference Z-scores of −2.5 SD. Her motor and cognitive development was mildly delayed. At age three years, her total IQ was 87. She attended a normal primary school and performed well. Microarray analysis showed a 1.3 Mb 22q11.2 deletion that was not detected in her parents.

Family 7

The female proband (Patient 15) was born at 36 weeks gestation with a birth weight of 1,785 g (−1.5 SD). She had an anteriorly placed anus, with an absent ventral part of the muscular ring of the sphincter. A cardiologic evaluation showed a large perimembranous ventricular septal defect, multiple muscular ventricular septal defects with left-to-right shunting, a large atrium septal defect type 2, and a wide and hypertrophic right ventricle. She had cyanotic attacks and feeding problems. Banding of the pulmonary artery was performed at age five months. Renal, myelum, and vertebral ultrasounds showed no additional abnormalities. Eleven rib pairs were seen on thorax radiographs. At six months of age, she was small, with length, weight, and head circumference Z-scores of −2.5 to −3 SD. Her motor development was mildly delayed and she was unable to turn from her back to her front. She had a square face with a broad forehead, hypertelorism, short palpebral fissures, low-set ears, a small umbilical hernia, and bilateral mild 2–3 syndactyly of

the toes. Microarray analysis showed a 1.1 Mb 22q11.2 deletion, which was absent in her parents.

Family 8

The proband (Patient 16) was referred for a genetic evaluation at the age of 12 years because of an unexplained proportionate short stature. His height Z-score and weight-for-height Z-score were −2.1 SD and −2.0 SD, respectively. His sitting height-to-height ratio and head circumference Z-scores were both −1.1 SD. He had a slender build and a triangular asymmetric face. One of his canines was missing. A single palmar crease was present on both his hands. He had dyslexia, but his motor and cognitive development were otherwise normal. His serum IGF-I and IGF-BP3 concentrations were repeatedly low, and a hand X-ray showed a delay in bone development of approximately 1.3 years. A cardiologic evaluation showed no abnormalities. He had a normal male karyotype (46,XY) and an analysis of the *SHOX* gene was also normal. Microarray analysis showed a 918 kb 22q11.2 deletion, which was not detected in his mother. His father was not available for genetic testing.

Family 9

The proband (Patient 17) was referred to a clinical geneticist at the age of 18 years because of behavioral problems in combination with a mild cognitive impairment. He was delivered by Caesarean at 38 weeks gestation after an uncomplicated pregnancy. His birth weight was 3,500 g (+1 SD). Although he started laughing at a normal age, all other developmental milestones during his first two years of live were delayed. He was able to sit without support at age 18 months and started to walk unaided at age two years. His speech development was also delayed and he had serious sleeping problems. Social interactions with his parents and friends were normal. After three years of nursery school he attended special primary and secondary schools for children with learning and behavioral problems. At age nine years, he was diagnosed with ADHD for which he received medication. Seven years later, he was also diagnosed with PDD-NOS. His total IQ score was 65. He performed relatively well in a quiet and structured environment. Apart from frequent middle ear infections during childhood, he had no complaints regarding his physical condition. His height was 182.8 cm (0 SD), weight 65.3 kg (0 SD), and head circumference 57.5 cm (0 SD). He had an asymmetrical face with a mild ptosis, full eyelids, low-set ears, long columella, narrow mouth with full lips, mild retrognathia, and a high palate with a normal uvula. His fingers were long and slender. A mild contracture of the left elbow was present. Molecular analysis of the *FMR1* gene revealed a normal (CGG)n-repeat length. Microarray analysis showed a 719 kb 22q11.2 deletion, which was not detected in his parents. Family history revealed that his sister had dyslexia and dyscalculia, and that his father also had PDD-NOS with a borderline intelligence. Several paternal family members had limited communication and social skills.

Family 10

The proband (Patient 18) was born at term with a birth weight of 3,750 g (+0.75 SD). He had spina bifida and macrocephaly secondary to ventriculomegaly. A brain MRI revealed an abnormal brain with a complete cerebellar agenesis, an extraordinary and rare finding. Additionally, he had a severe scoliosis and a slightly dysmorphic appearance characterized by epicanthic folds and hypertelorism. At the age of four years he was severely handicapped, requiring full time care and was unable to recognize his parents. He had a normal male karyotype (46,XY). Microarray analysis showed a 401 kb 22q11.2 deletion. The same deletion was detected in the asymptomatic mother (patient 19). The parents were not consanguineous and their family history was normal apart from a paternal first cousin with spina bifida.

Family 11

The female proband (Patient 20) was the first child of nonconsanguineous parents. During the 27th week of pregnancy, growth retardation and placental insufficiency was detected. She was delivered by Caesarean at 31 weeks gestation. Her birth weight was 775 g (−2 SD) and she had a mild bronchial dysplasia. A cardiac evaluation revealed a small muscular ventricular septal defect and an atrium septal defect type 2. During childhood she suffered from frequent upper respiratory tract infections. At the age of eight years, she received speech therapy for stuttering and to improve her pronunciation. She attended a regular school, but needed extra support. Her motor development was normal. At physical examination, she had a height of 114 cm (−3.5 SD), a weight of 15.6 kg (−3.0 SD), and a head circumference of 46.5 cm (−3.3 SD). Her appearance was characterized by a triangular face with a small forehead, arched eyebrows, upslanting palpebral fissures, posterior rotated ears with attached lobes, a nose with a high narrow nasal bridge and a long columella, and a small mouth with thin vermilion of the lips. She had irregular teeth with fusion of the two right lower incisors. Her thorax was bell-shaped and she had an umbilical hernia. She had hypermobile joints, fetal finger pads, and a sandal gap between her first and second toes. Microarray analysis showed a 1.3 Mb 22q11.2 deletion that was not observed in her parents.

Family 12

The male proband (Patient 21) was born to nonconsanguineous parents. He was delivered by Caesarean at 37 weeks gestation. His birth weight was 2,590 g (−1 SD). He was hypotonic and his early motor development was delayed. At two years of age, a non-progressive cerebral palsy was diagnosed. His head circumference Z-score was 0 SD. He had no dysmorphic facial features. A neurological examination showed a mild right-sided hemiplegia with dystonic and choreatic movements. MRI scans of his brain showed periventricular white matter changes without any other abnormalities. A prenatal or perinatal insult was suspected. An EEG and a cardiologic evaluation were normal. At age 11 years, he had borderline intelligence and difficulties with social interactions. He attended a small class in a normal school. A diagnosis of PDD-NOS and an autism spectrum disorder were the reason for his referral to the genetics clinic. He had a normal male karyotype (46,XY). Microarray analysis showed a 414 kb 22q11.2 deletion. The same deletion was detected in the asymptomatic mother

(Patient 22). There was no family history of neurological disorders, autism or congenital anomalies.

Family 13

A 31-year-old female (Patient 23) visited the outpatient genetics clinic for counseling because of short stature and learning problems. She was born at 36 weeks gestation with a birth weight of 1,430 g (−2 SD) and a small placenta. She remained short during childhood, had frequent ear infections, and attended a special secondary school for children with learning problems. Her height at age 31 years was 153 cm (−2.7 SD) and her head circumference was 53 cm (−1.3 SD). She had a high forehead, mild ptosis and epiblepharon, thin vermilion of the upper lip, everted thick vermilion of the lower lip, with a normal palate and uvula. Her fingers and toes were short, and she had a partial cutaneous syndactyly of the second and third toes. Microarray analysis showed a 1.4 Mb 22q11.2 deletion. The deletion was not present in her parents.

Family 14

The male proband (Patient 24) was born after a pregnancy complicated by intra-uterine growth restriction and reduced fetal movements. He was delivered by Caesarean after 36 weeks gestation. His birth weight was 1,569 g (−1.8 SD) and his birth length was 43 cm (−1.3 SD). At birth, an anal atresia, umbilical hernia, diastasis recti and a left sided renal agenesis were discovered. His right kidney was hyperechogenic and enlarged. He had no heart anomaly or cleft palate. His development was delayed: he first walked at the age of three years and started to talk around three to four years. He attended a special school for children with learning problems. At age 10 years, he had a mild to moderate intellectual disability. His height was 133 cm (−2 SD) and his head circumference was 51.5 cm (−1 SD). He had bilateral pre-auricular tags, mild dysplastic ears with over-folded helices, a strabismus convergens alternans, and a slight facial asymmetry. The end phalanges of his fingers and toes were relatively short and his first toes were broad. In addition, he had a maldescensus of his right testis. Chromosome analysis showed a balanced inversion of chromosome 9 (46,XY,inv[9][p11q13]). Microarray analysis showed a 1.2 Mb 22q11.2 deletion, which was not detected in his mother. His father was not available for genetic testing.

Family 15

The female proband (Patient 25) was born by vacuum extraction at 35 weeks gestation. Her birth weight was 2,410 g (0 SD) and birth length was 44 cm (0 SD). She had a hip dysplasia, which was treated with an abduction brace and traction. Her development was mildly delayed. She first stood when she was 14 months old and walked unsupported from the age of 19 months. She received both physical and speech therapy. She also had behavioral problems, characterized by hyperactivity, anxiety, and sometimes auto-mutilation. She had frequent middle ear infections and several episodes of staring, but no formal diagnosis of epilepsy was made. A cardiac evaluation was normal and she had no renal anomalies. At three years of age, her height was 93.5 cm (−1.75 SD) and her head circumference was 46.8 cm (−1.75 SD). She had down slanting palpebral fissures, a mild ptosis of the eyelids, smooth philtrum, and a thin vermilion of the upper lip. She further had general increased joint mobility and a hyperlordosis. The mother of the proband was also treated for hip dysplasia and had some learning difficulties as a child. The father of the proband (Patient 26) was evaluated for growth hormone deficiency because of short stature. His current height was 162 cm (−3 SD). During childhood he had also had behavioral problems with hyperactivity and temper tantrums. His motor and cognitive development were further normal. During adulthood he was treated for depression and psychosis. A cardiologic evaluation was normal. The paternal grandmother (Patient 27) had had two episodes of psychotic behavior, for which she was treated in a psychiatric clinic. Microarray analysis showed a 786 kb 22q11.2 deletion in the proband. The same deletion was detected in her father and paternal grandmother.

RESULTS

A schematic representation of the deletions found in the 15 families presented in this study is shown in Figure 1. Seven deletions involved LCR22-B and LCR22-D (B to D type deletions), five deletions involved LCR22-C and LCR22-D (C to D type deletions), and three deletions also involved LCR22-C, but extended beyond LCR22-D (C to beyond D type deletions). The estimated sizes of these deletions varied from approximately 400 kb to 1.4 Mb (Table I).

In 12 families, both parents of the proband were available for genetic analysis. Five (42%) of the deletions occurred de novo in the proband, and seven (58%) of the deletions were inherited (four paternally and three maternally). In the remaining three families, the inheritance was unknown. Maternal inheritance could be excluded in two of these families, and paternal inheritance was excluded in the other family. In addition to the parents, other family members were investigated in three families with an inherited deletion. In family 1, analysis of the grandparents showed that the deletion had occurred de novo in the father of the proband.

In total, there were 27 patients with a central 22q11.2 deletion in these 15 families. The clinical features of all 27 patients are summarized in Table II. The clinical findings reported in 25 previously described patients with central 22q11.2 deletions are summarized in Table S1 (see supporting information online) [Kurahashi et al., 1997; Garcia-Minaur et al., 2002; Rauch et al., 2005; D'Angelo et al., 2007; Fernandez et al., 2009; Ogilvie et al., 2009; Garavelli et al., 2011; Ledig et al., 2011; Yu et al., 2011; Breckpot et al., 2012; Sanna-Cherchi et al., 2012; Verhagen et al., 2012]. The frequency of reported features in all the currently identified patients in relation to the deletion types is reviewed in Table III.

In patients with a central B to D type or a C to D type deletion, developmental problems, including learning problems, speech delay, motor delay or (mild) cognitive impairment, were common and occurred in 25 patients (54% of total). Fourteen (30%) of these deletion carriers had psychiatric or behavioral problems, including attention deficit, hyperactivity, or a pervasive/autism spectrum disorder. Craniofacial features were present in 33 (72%) of the deletion carriers. The most frequent reported facial features were a triangular asymmetric face, a broad forehead, short upslanting palpebral fissures, ptosis of the eyelids, thin vermilion of the

TABLE II. Clinical Findings in Patients with Central 22q11.2 Deletions

Family	Family 1			Family 2		Family 3	Family 4	
Patient	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Deletion type	B to D	B to D	B to D	C to D	C to D	B to D	C to D	C to D
Inheritance	Paternal	Paternal	De novo	Maternal	Unknown	Unknown	Paternal	Paternal
Age	3 years	prenatal	30 years	7 years	31 years	4 years	8 years	38 years
Sex	Male	Male	Male	Male	Female	Male	Male	Male
Growth delay	–	–	–	–	–	–	–	–
Microcephaly	–	–	–	–	–	–	–	–
Development	Speech delay		Learning problems, dyslexia	-	Mild cognitive impairment	Mild cognitive impairment, speech delay, motor delay	Mild cognitive impairment, dyslexia	Learning problems
Behavior	–		–	–	–	ADHD, PDD-NOS, aggression	ADD, anxiety disorder, aggression	–
Craniofacial features	Triangular face, broad forehead, high nasal bridge, upslanting palpebral fissures	Micrognathia, low-set ears, overfolded helices	Asymmetric face, broad forehead, upslanting palpebral fissures, deep set eyes	Thin vermilion upper lip, overfolded helix	Square face, upslanting palpebral fissures, overfolded helices	Triangular asymmetric face, broad forehead, open mouth, full lower lip	Hypotonic face, open mouth, high narrow palate, epicanthic folds, peri-orbital fullness, ptosis	–
Heart anomaly	–	–	–	–	Atrio-ventricular septum defect, mitral valve incompetence	–	–	–
Urogenital anomaly	Renal agenesis	Horseshoe kidney, renal cysts, bladder septum	Renal dysplasia, ectopic ureter, bladder diverticulum	Renal agenesis, cryptorchid testis, ductus deferens agenesis	–	–	Mild hydronephrosis	Mild hydronephrosis
Ear infections	+	–	–	–	–	+	+	–
Skeletal abnormality	–	Club feet	Mild scoliosis	–	Kyphosis, pes cavus	Overriding toes	–	–
Other features				Inguinal hernia	Spasticity, increased reflexes, tremor	Conductive hearing loss	Nasal speech, increased reflexes, tremor	Tremor

Family	Family 4			Family 5		Family 6	Family 7
Patient	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Deletion type	C to D	C to D	C to D	B to D	B to D	C to beyond D	B to D
Inheritance	Paternal	Paternal	Unknown	Paternal	Unknown	De novo	De novo
Age	5 years	13 years	68 years	16 years	67 years	15 months	6 months
Sex	Male	Male	Male	Male	Male	Female	Female
Growth delay	−	+	−	−	−	+	+
Microcephaly	−	−	−	−	−	+	+
Development	Speech delay, motor delay	Delayed, dyslexia	−	−	−	Mild delay	Motor delay
Behavior	−	PDD-NOS	−	ADD	−	−	−
Craniofacial features	−	Triangular face	Long face, short palpebral fissures, high narrow palate	Long face, short palpebral fissures, flat malar region, earlobe pits, high palate, absent nasal cartilage	−	Flat face, small alae nasi, thin vermillion of upper lip, overfolded helices	Square face, broad forehead, hypertelorism, short palpebral fissures, low-set ears
Heart anomaly	−	−	Ventricular extra systoles	Perimembranous ventricular septal defect	−	Ventricular septal defect, overriding aorta	Perimembranous ventricular septal defect, muscular ventricular septal defects, atrium septal defect type 2
Urogenital anomaly	Phymosis, uteropelvic junction stenosis	−	Single renal cyst	IgM nephropathy, urethral meatus stenosis	−	−	−
Ear infections	−	−	−	+	−	−	−
Skeletal abnormality	−	Long fingers	−	Pectus excavatum, long fingers, 2–3 syndactyly of toes	−	Tapering fingers, clinodactyly	11 rib pairs, 2–3 syndactyly of toes
Other features		Neonatal hypotonia and feeding problems	Diaphragmatic hernia, tremor	Inguinal hernia, diastasis recti, missing molar,	Asymptomatic	Hydro-syringomyelia, mild immuno-deficiency	Anal atresia, umbilical hernia, neonatal feeding problems

TABLE II. (*Continued*)

Family	Family 8	Family 9	Family 10		Family 11	Family 12		Family 13
Patient	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
Deletion type	C to D	B to D	C to D	C to D	C to beyond D	C to D	C to D	C to beyond D
Inheritance	Unknown	De novo	Maternal	Unknown	De novo	Maternal	Unknown	De novo
Age	12 years	18 years	4 years	(adult)	8 years	11 years	(adult)	31 years
Sex	Male	Male	Male	Female	Female	Male	Female	Female
Growth delay	+	−	−		+	−		+
Microcephaly	−	−	−		+	−		−
Development	Dyslexia	Delayed, mild cognitive impairment	Severe motor and cognitive impairment		Learning problems	Motor delay, borderline intelligence		Learning problems
Behavior	−	ADHD, PDD-NOS			−	PDD-NOS		−
Craniofacial features	Triangular asymmetric face	Asymmetric face, mild ptosis, low-set ears, long collumnella, full lips, high palate	Macrocephaly, epicanthal folds, hypertelorism		Triangular face, small forehead, arched eyebrows, upslanting palpebral fissures, high nasal bridge, thin vermillion of upper lip	−		High forehead, mild ptosis, epiblepharon, thin vermillion upper lip, thick vermillion lower lip
Heart anomaly	−	−	−		Ventricular septal defect, atrium septal defect type 2	−		−
Urogenital anomaly	−	−	−		−	−		−
Ear infections	−	+	−		+	−		+
Skeletal abnormality	−	Long fingers	Scoliosis		Bell-shaped thorax, sandal gap	−		Short fingers and toes
Other features	Missing canine, single palmar creases		Spina bifida, hydrocephaly, cerebellum agenesis	Asymptomatic	Umbilical hernia, fusion of incisors	Neonatal hypotonia, cerebral palsy	Asymptomatic	Cutaneous syndactyly 2^{nd}–3^{rd} toes

Family	Family 14	Family 15		
Patient	**24**	**25**	**26**	**27**
Deletion type	B to D	B to D	B to D	B to D
Inheritance	Unknown	Paternal	Maternal	Unknown
Age	10 years	3 years	39 years	75 years
Sex	Male	Female	Male	Female
Growth delay	IUGR	–	short stature	
Microcephaly	–	–	–	–
Development	Delayed, mild cognitive impairment	Delayed	Normal	
Behavior		Hyperactivity, anxiety, auto-mutilation	Hyperactivity, depression, psychosis	Psychosis
Craniofacial features	Asymetric face, ptosis, strabismus, ear tags, over-folded helices	Down slanting palpebral fissures, ptosis, thin vermillion upper lip		
Heart anomaly	–	–	–	
Urogenital anomaly	Renal agenesis	–		
Ear infections		+		
Skeletal abnormality	Short fingers and toes	Joint mobility, hyperlordosis		
Other features	Anal atresia Umbilical hernia Diastasis recti	Hip dysplasia		

ADHD, attention deficit hyperactivity disorder. ADD, attention deficit disorder. PDD-NOS, pervasive developmental disorder not otherwise specified.

TABLE III. Frequency of Reported Features in Patients with Central 22q11.2 Deletions

Deletion type	Central B to D			Central C to D				Central to distal C to beyond D			Common 22q11.2 deletion[b]	Distal 22q11.2 deletion[c]
	This study	Literature[a]	Combined	This study	Literature[a]	Combined	All	This study	Literature[a]	Combined		
Number of patients	12	5	17	12	17	29	46	3	3	6		
Growth delay	3	1	4 (24%)	2	4	6 (21%)	10 (22%)	3	3	6 (100%)	36%	82%
Microcephaly	1	1	2 (12%)	0	3	3 (10%)	5 (11%)	2	3	5 (83%)	10%	68%
Developmental delay and/or cognitive impairment	7	2	9 (53%)	7	9	16 (55%)	25 (54%)	3	1	4 (66%)	38-68%	94%
Behavioral problems and/or psychiatric disorder	6	1	7 (41%)	3	4	7 (24%)	14 (30%)	0	1	1 (17%)	14-54%	24%
Dysmorphic craniofacial features	9	5	14 (82%)	7	12	19 (66%)	33 (72%)	3	2	5 (83%)	NR	40%
Congenital heart anomaly	2	2	4 (24%)	1	3	4 (14%)	8 (17%)	2	3	5 (83%)	75-77%	41-71%
Cleft palate	0	0	0	0	0	0	0	0	0	0	11-16%	23%
Urinary tract or genital anomaly	5	0	5 (29%)	5	6	11 (38%)	16 (35%)	0	0	0	36%	4-5%
Ear infections	5	1	6 (35%)	1	1	2 (7%)	8 (17%)	2	0	2 (33%)	25-33%	NR
Inherited deletion[d]	3/5	2/2	5/7 (71%)	4/4	3/5	7/9 (78%)	12/16 (76%)	0/3	0/2	0/5 (0%)	6-10%	17%

NR, not reported.

[a]Based on Kurahashi et al. [1997]; Garcia-Minaur et al. [2002]; Rauch et al. [2005]; D'Angelo et al. [2007]; Fernandez et al. [2009]; Ogilvie et al. [2009]; Garavelli et al. [2011]; Ledig et al. [2011]; Yu et al. [2011]; Breckpot et al. [2012]; Sanna-Cherchi et al. [2012]; Verhagen et al. [2012]. See supporting information online for more details on previously published cases.

[b]Common 22q11.2 deletion syndrome, deletions from LCR22-A to LCR22-D, based on Ryan et al. [1997] and McDonald-McGinn and Sullivan [2011].

[c]Distal 22q11.2 deletion syndrome, deletions distal from LCR22-D, based on Fagerberg et al. [2013].

[d]Inheritance was considered in the probands only.

upper lip, low-set ears, and overfolded helices. Photographs of deletion carriers, who consented to their publication, are shown in Figure 2. Although several facial features were shared among deletion carriers, the facial phenotype does not seem to be particularly distinctive or recognizable. Congenital anomalies of the kidneys or urinary tract were reported in 35% of the patients with a central 22q11.2 deletion. None of the patients had a cleft palate. Three subjects with these central 22q11.2 deletions were reported to be asymptomatic.

Patients with a central to distal deletion, a deletion that extended beyond LCR22-D, had a more severe phenotype. The frequency of congenital heart anomalies was relatively high (83%) in these patients, when compared to patients with a central B to D type or a C to D type deletion (24% and 14%, respectively) (Table III). Growth restriction, short stature and microcephaly were also more frequent in patients with a C to beyond D type deletion. All C to beyond D type deletions occurred de novo, whereas 71–78% of the B to D type and C to D type deletions were inherited.

DISCUSSION

Central 22q11.2 deletions are associated with a highly variable, and difficult to recognize, clinical phenotype with many features that are shared with the common ~3 Mb 22q11.2 deletion syndrome.

FIG. 2. Clinical photographs of patients with a central 22q11.2 deletion. Photographs of Patient 1 (at age 3 years), Patient 3 (at age 30 years), Patient 4 (at age 7 years), Patient 5 (at age 31 years), Patient 6 (at age 8 years), Patient 12 (at age 16 years), Patient 15 (at age 6 months), Patient 20 (at age 8 years), Patient 23 (at age 31 years), Patient 24 (at age 10 years) and Patient 25 (at age 3 years). The facial phenotype is not particularly distinctive or recognizable. [Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833]

As is common in family studies on microdeletion syndromes, the overall phenotype is milder or more normal in family members than in the index cases. Thus, the penetrance of the deletion is probably overestimated, reflecting an ascertainment bias.

The majority of the central 22q11.2 deletions resulted from recombination between either LCR22-B or LCR22-C, and LCR-D. In three families, however, a deletion was found that extended beyond LCR22-D and that overlapped with the proximal part of the distal 22q11.2 deletion syndrome region. Very similar deletions have been reported before [Ogilvie et al., 2009; Garavelli et al., 2011; Breckpot et al., 2012]. These deletions are associated with distal breakpoint sites that do not correspond to one of the known LCR22 blocks. It has been shown that other blocks of high sequence homology may serve as alternative recombination substrates [Breckpot et al., 2012]. The clinical phenotype in these patients is distinct from the other patients, and shares more characteristics with the distal 22q11.2 deletion syndrome, in which a history of prematurity, prenatal and postnatal growth delay and microcephaly are also prevalent [Ben-Shachar et al., 2008; Fagerberg et al., 2013].

Inheritance was investigated in nine of the previously reported probands with a central 22q11.2 deletion (see supporting information online) [Garcia-Minaur et al., 2002; D'Angelo et al., 2007; Fernandez et al., 2009; Ogilvie et al., 2009; Garavelli et al., 2011; Verhagen et al., 2012]. Five (56%) patients inherited the deletion – two paternally and three maternally – and four deletions had occurred de novo. In our cohort, 7/12 (58%) of the deletions were inherited by the probands. Combined, 12/21 (57%) of the central 22q11.2 deletions in the probands – and 17/29 (57%) of the central 22q11.2 deletions in all cases – were inherited from a parent, with an equal distribution between paternal and maternal transmission to offspring.

The high frequency of familial deletions we observe is in contrast to the high rate of de novo deletions (approximately 90–94%) seen among patients with the common ~3 Mb 22q11.2 deletion [McDonald-McGinn and Sullivan, 2011]. Similarly, previous studies demonstrated that the shorter nested ~1.5 Mb proximal deletion also occurs more frequently among familial cases with 22q11.2 deletions [Adeyinka et al., 2004; Fernandez et al., 2005]. To try and explain this difference, it was suggested that patients with the smaller proximal 22q11.2 deletion may have a better fecundity than patients with the larger ~3 Mb common deletion, and that genes within the LCR22-B to LCR22-D interval might be involved in reproductive functions such as spermatogenesis [Fernandez et al., 2005]. However, our observation of a high familial occurrence of B to D type and C to D type 22q11.2 deletions does not support this hypothesis, but does support the notion that smaller 22q11.2 deletions might be better tolerated than larger deletions. Differences in the severity of behavioral problems, psychiatric disorders and cognitive impairment (as example) could explain the affected individual having a better chance of producing offspring [Bartsch et al., 2003].

We observed a lower frequency of cardiac anomalies in patients with central 22q11.2 deletions that were located between LCR22-B and LCR22-D, as compared to reported frequencies in patients with the larger ~3 Mb common deletion or a distal 22q11.2 deletion. Cardiovascular anomalies were reported in 8/46 (17%) of the patients with a B to D type or a C to D type deletion. The most common anomalies were atrial and ventricular septal defects. Among the previously reported cases, three patients had a tetralogy of Fallot and one patient had a hypoplastic right heart with a double outlet right ventricle. Truncus arteriosus was seen in two of the patients with a deletion that extended beyond LCR22-D. In comparison, heart defects are reported in 75–77% of patients with a common ~3 Mb 22q11.2 deletion [Ryan et al., 1997; McDonald-McGinn and Sullivan, 2011], and in 41–71% of published patients with a distal 22q11.2 deletion [Fagerberg et al., 2013].

Three genes (*TBX1*, *CRKL*, and *MAPK1*) on 22q11.2 have been identified whose haploinsufficiency is considered causative for the congenital heart defects in 22q11.2 deletion syndrome [Lindsay, 2001; Momma, 2010]. Loss of *TBX1* occurs in the proximal and common 22q11.2 deletions. Loss of *CRKL* occurs in the common and central 22q11.2 deletions. And lastly, loss of *MAPK1* occurs in distal 22q11.2 deletions and in central 22q11.2 deletions that extend beyond LCR22-D (C to beyond D type deletions).

One could speculate that the larger ~3 Mb common deletion, with a combined loss of the genes *TBX1* and *CRKL*, could be more deleterious than the smaller proximal or central 22q11.2 deletions with loss of either *TBX1* or *CRKL*. In mice, compound heterozygosity of both Crkl and Tbx1 induces a higher penetrance of thymus defects, parathyroid defects, and cardiovascular defects than heterozygosity of Crkl or Tbx1 alone [Guris et al., 2006]. Homozygous loss of the mitogen-activated protein kinase 1 (*ERK2/MAPK1*) gene in a murine model results in ventricular septal defects and conotruncal heart abnormalities [Newbern et al., 2008]. It is believed that the hemizygous loss of this gene contributes to the congenital heart anomalies seen in patients with a distal 22q11.2 deletion [Fagerberg et al., 2013]. The recurrent central 22q11.2 deletion that overrides LCR22-D contains both the *MAPK1* gene as well as the *CRKL* gene. Five of the six patients (83%) with this recurrent deletion presented with a congenital heart defect. It may be that the combined deletion of these two genes results in a higher penetrance of cardiovascular anomalies than the loss of either gene alone, similar to the previously suggested interaction between *TBX1* and *CRKL* [Guris et al., 2006].

TBX1, *CRKL*, and *MAPK1* are all involved in a common genetic pathway regulating heart outflow tract morphogenesis [Moon et al., 2006; Vallejo-Illarramendi et al., 2009]. It is conceivable that dosage-dependent perturbations in the expression of these genes may contribute to the penetrance of congenital heart defects in subjects with 22q11.2 deletions. This could explain why patients with loss of two of these genes – i.e., patients with the common ~3 MB deletion with loss of *TBX1* and *CRKL*, or patients with a central deletion overriding LCR22-D with loss of *CRKL* and *MAPK1*–have the highest incidence of heart defects. All five deletions overriding LCR22-D in which inheritance could be studied occurred de novo, which further suggests that these overriding deletions are less well tolerated than the smaller central 22q11.2 deletions with loss of *CRKL* alone.

The observed renal and urogenital anomalies in central 22q11.2 deletion carriers are similar to and occurred with equal overall frequencies to those reported in carriers of the ~3 Mb common deletion [Ryan et al., 1997; Stewart et al., 1999; Wu et al., 2002; Kujat

et al., 2006]. The smallest deleted segment in subjects with an urogenital anomaly was the region between LCR22-C and LCR22-D. In two recent studies, C to D type central 22q11.2 deletions were found in three out of 192 individuals with isolated renal hypodysplasia and in 1 out of 56 patients with Mayer-Rokitansky-Hauser syndrome [Ledig et al., 2011; Sanna-Cherchi et al., 2012]. This suggests that a gene responsible for this part of the phenotype is located within the central 22q11.2 deletion region [Sanna-Cherchi et al., 2012]. To our knowledge, no mutation of any gene in this particular region has been associated with congenital renal and urinary tract malformations yet. Although it does not exclude involvement of the *CRLK* gene, renal and urogenital anomalies were not reported in Crlk-deficient mice [Guris et al., 2001; Moon et al., 2006]. Another candidate gene related to this part of the phenotype could be the leucine zipper-like transcriptional regulator 1 gene (*LZTR1*; OMIM 600574). This putative transcription factor is expressed in the kidney and is thought to be involved in embryogenesis.

A high palate was reported in four patients with a central 22q11.2 deletion. Nasal speech was reported only in two cases. None of the 52 patients with a central 22q11.2 deletion had a cleft palate. In patients with the common ~3 Mb deletion, a cleft palate or velopharyngeal insufficiency occurs in approximately 11–16% and 42% of cases, respectively [McDonald-McGinn and Sullivan, 2011; Ryan et al., 1997]. Thus, it seems that cleft palate or velopharyngeal insufficiency is uncommon among central 22q11.2 deletion carriers.

None of the central 22q11.2 deletion carriers presented with hypocalcemia, hypoparathyroidism or a clinical phenotype of DiGeorge anomaly, but recurrent ear infections occurred in 17% of the patients, and a mild immunodeficiency with diminished number of T-cells and increased number natural killer cells was reported in one patient of our cohort. Elevated natural killer cells and abnormal T cell subpopulations were also reported in one previous case [D'Angelo et al., 2007; de Queiroz Soares et al., 2012]. It has been suggested that the genes *SNAP29*, *LZTR1*, and/or *P2RXL1* could be important for immune regulation [de Queiroz Soares et al., 2012].

One patient with a central 22q11.2 deletion in our cohort had a severe cognitive impairment with spina bifida and a complete cerebellum agenesis. Severe cognitive impairments, spina bifida and developmental abnormalities of the cerebellum, including cerebellar hypoplasia or dysgenesis, have been reported before but are rarely seen in patients with a common 22q11.2 deletion [Lynch et al., 1995; Devriendt et al., 1996; McDonald-McGinn and Sullivan, 2011]. We found that the asymptomatic mother of our patient carried the same deletion, and that spina bifida also occurred in a paternal family member, however, it remains uncertain to what extent the deletion contributed to the occurrence of these severe congenital anomalies. Since deletions of 22q11.2 are relatively common, they could occur in combination with other congenital disorders purely by chance alone.

In summary, central 22q11.2 deletions are associated with a variable and wide spectrum of clinical features, very similar to the features and variability seen in subjects that carry the more common ~3 Mb deletion on 22q11.2. Intellectual impairments, behavioral problems, facial dysmorphisms and urogenital anomalies seem to be equally common. Cleft palate, hypocalcemia, hypoparathyroidism, and DiGeorge sequence, however, have not been reported so far. Congenital heart anomalies are less frequent, except for the subgroup of deletion carriers in which the *MAPK1* gene is also lost. Microcephaly and growth restriction are also seen more frequently in association with this deletion type. Because of the overall overlap in clinical presentation we suggest that patients with a central 22q11.2 deletion should be assessed and offered the same type of management and support as other patients with 22q11.2 deletions [Bassett et al., 2011]. And since central 22q11.2 deletions are often inherited, parents of an index case should be offered deletion screening and appropriate genetic counseling.

ACKNOWLEDGMENTS

We thank all patients and their family members for their participation, and Kate McIntyre for editing the manuscript.

REFERENCES

Adeyinka A, Stockero KJ, Flynn HC, Lorentz CP, Ketterling RP, Jalal SM. 2004. Familial 22q11.2 deletions in DiGeorge/velocardiofacial syndrome are predominantly smaller than the commonly observed 3Mb. Genet Med 6:517–520.

Bartsch O, Nemeckova M, Kocarek E, Wagner A, Puchmajerova A, Poppe M, Ounap K, Goetz P. 2003. DiGeorge/velocardiofacial syndrome: FISH studies of chromosomes 22q11 and 10p14, and clinical reports on the proximal 22q11 deletion. Am J Med Genet A 117A:1–5.

Bassett AS, McDonald-McGinn DM, Devriendt K, Digilio MC, Goldenberg P, Habel A, Marino B, Oskarsdottir S, Philip N, Sullivan K, Swillen A, Vorstman J. International 22q11.2 Deletion Syndrome Consortium. 2011. Practical guidelines for managing patients with 22q11.2 deletion syndrome. J Pediatr 159:332–339.e1.

Ben-Shachar S, Ou Z, Shaw CA, Belmont JW, Patel MS, Hummel M, Amato S, Tartaglia N, Berg J, Sutton VR, Lalani SR, Chinault AC, Cheung SW, Lupski JR, Patel A. 2008. 22q11.2 distal deletion: A recurrent genomic disorder distinct from DiGeorge syndrome and velocardiofacial syndrome. Am J Hum Genet 82:214–221.

Breckpot J, Thienpont B, Bauters M, Tranchevent LC, Gewillig M, Allegaert K, Vermeesch JR, Moreau Y, Devriendt K. 2012. Congenital heart defects in a novel recurrent 22q11.2 deletion harboring the genes CRKL and MAPK1. Am J Med Genet A 158A:574–580.

Carlson C, Sirotkin H, Pandita R, Goldberg R, McKie J, Wadey R, Patanjali SR, Weissman SM, Anyane-Yeboa K, Warburton D, Scambler P, Shprintzen R, Kucherlapati R, Morrow BE. 1997. Molecular definition of 22q11 deletions in 151 velo-cardio-facial syndrome patients. Am J Hum Genet 61:620–629.

D'Angelo CS, Jehee FS, Koiffmann CP. 2007. An inherited atypical 1 mb 22q11.2 deletion within the DGS/VCFS 3 mb region in a child with obesity and aggressive behavior. Am J Med Genet A 143A:1928–1932.

de Queiroz Soares DC, Dutra RL, D'angioli Costa Quaio CR, Melaragno MI, Kulikowski LD, Torres LC, Kim CA. 2012. Role of SNAP29, LZTR1 and P2RXL1 genes on immune regulation in a patient with atypical 0.5 mb deletion in 22q11.2 region. Clin Immunol 145:55–58.

de Vries BB, Pfundt R, Leisink M, Koolen DA, Vissers LE, Janssen IM, Reijmersdal S, Nillesen WM, Huys EH, Leeuw N, Smeets D, Sistermans EA, Feuth T, van Ravenswaaij-Arts CM, van Kessel AG, Schoenmakers EF, Brunner HG, Veltman JA. 2005. Diagnostic genome profiling in mental retardation. Am J Hum Genet 77:606–616.

Devriendt K, Thienen MN, Swillen A, Fryns JP. 1996. Cerebellar hypoplasia in a patient with velo-cardio-facial syndrome. Dev Med Child Neurol 38:949–953.

Edelmann L, Pandita RK, Spiteri E, Funke B, Goldberg R, Palanisamy N, Chaganti RS, Magenis E, Shprintzen RJ, Morrow BE. 1999. A common molecular basis for rearrangement disorders on chromosome 22q11. Hum Mol Genet 8:1157–1167.

Emanuel BS. 2008. Molecular mechanisms and diagnosis of chromosome 22q11.2 rearrangements. Dev Disabil Res Rev 14:11–18.

Fagerberg CR, Graakjaer J, Heinl UD, Ousager LB, Dreyer I, Kirchhoff M, Rasmussen AA, Lautrup CK, Birkebaek N, Sorensen K. 2013. Heart defects and other features of the 22q11 distal deletion syndrome. Eur J Med Genet 56:98–107.

Fernandez L, Lapunzina P, Pajares IL, Criado GR, Garcia-Guereta L, Perez J, Quero J, Delicado A. 2005. Higher frequency of uncommon 1.5-2 mb deletions found in familial cases of 22q11.2 deletion syndrome. Am J Med Genet A 136:71–75.

Fernandez L, Nevado J, Santos F, Heine-Suner D, Martinez-Glez V, Garcia-Minaur S, Palomo R, Delicado A, Pajares IL, Palomares M, Garcia-Guereta L, Valverde E, Hawkins F, Lapunzina P. 2009. A deletion and a duplication in distal 22q11.2 deletion syndrome region. Clinical implications and review. BMC Med Genet 10:48.

Garavelli L, Rosato S, Wischmeijer A, Gelmini C, Esposito A, Mazzanti L, Franchi F, De Crescenzo A, Palumbo O, Carella M, Riccio A. 2011. 22q11.2 distal deletion syndrome: Description of a new case with truncus arteriosus type 2 and review. Mol Syndromol 2:35–44.

Garcia-Minaur S, Fantes J, Murray RS, Porteous ME, Strain L, Burns JE, Stephen J, Warner JP. 2002. A novel atypical 22q11.2 distal deletion in father and son. J Med Genet 39:E62.

Guris DL, Duester G, Papaioannou VE, Imamoto A. 2006. Dose-dependent interaction of Tbx1 and crkl and locally aberrant RA signaling in a model of del22q11 syndrome. Dev Cell 10:81–92.

Guris DL, Fantes J, Tara D, Druker BJ, Imamoto A. 2001. Mice lacking the homologue of the human 22q11.2 gene CRKL phenocopy neurocristopathies of DiGeorge syndrome. Nat Genet 27:293–298.

Hehir-Kwa JY, Egmont-Petersen M, Janssen IM, Smeets D, van Kessel AG, Veltman JA. 2007. Genome-wide copy number profiling on high-density bacterial artificial chromosomes, single-nucleotide polymorphisms, and oligonucleotide microarrays: A platform comparison based on statistical power analysis. DNA Res 14:1–11.

Kujat A, Schulz MD, Strenge S, Froster UG. 2006. Renal malformations in deletion 22q11.2 patients. Am J Med Genet A 140:1601–1602.

Kurahashi H, Tsuda E, Kohama R, Nakayama T, Masuno M, Imaizumi K, Kamiya T, Sano T, Okada S, Nishisho I. 1997. Another critical region for deletion of 22q11: A study of 100 patients. Am J Med Genet 72: 180–185.

Ledig S, Schippert C, Strick R, Beckmann MW, Oppelt PG, Wieacker P. 2011. Recurrent aberrations identified by array-CGH in patients with Mayer-Rokitansky-Kuster-Hauser syndrome. Fertil Steril 95:1589–1594.

Lindsay EA. 2001. Chromosomal microdeletions: Dissecting del22q11 syndrome. Nat Rev Genet 2:858–868.

Lynch DR, McDonald-McGinn DM, Zackai EH, Emanuel BS, Driscoll DA, Whitaker LA, Fischbeck KH. 1995. Cerebellar atrophy in a patient with velocardiofacial syndrome. J Med Genet 32:561–563.

McDonald-McGinn DM, Sullivan KE. 2011. Chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). Medicine (Baltimore) 90:1–18.

Mikhail FM, Descartes M, Piotrowski A, Andersson R, Diaz de Stahl T, Komorowski J, Bruder CE, Dumanski JP, Carroll AJ. 2007. A previously unrecognized microdeletion syndrome on chromosome 22 band q11.2 encompassing the BCR gene. Am J Med Genet A 143A: 2178–2184.

Momma K. 2010. Cardiovascular anomalies associated with chromosome 22q11.2 deletion syndrome. Am J Cardiol 105:1617–1624.

Moon AM, Guris DL, Seo JH, Li L, Hammond J, Talbot A, Imamoto A. 2006. Crkl deficiency disrupts Fgf8 signaling in a mouse model of 22q11 deletion syndromes. Dev Cell 10:71–80.

Newbern J, Zhong J, Wickramasinghe RS, Li X, Wu Y, Samuels I, Cherosky N, Karlo JC, O'Loughlin B, Wikenheiser J, Gargesha M, Doughman YQ, Charron J, Ginty DD, Watanabe M, Saitta SC, Snider WD, Landreth GE. 2008. Mouse and human phenotypes indicate a critical conserved role for ERK2 signaling in neural crest development. Proc Natl Acad Sci U S A 105:17115–17120.

Ogilvie CM, Ahn JW, Mann K, Roberts RG, Flinter F. 2009. A novel deletion in proximal 22q associated with cardiac septal defects and microcephaly: A case report. Mol Cytogenet 2:9.

Óskarsdóttir S, Vuijc M, Fasth A. 2004. Incidence and prevalence of the 22q11 deletion syndrome: A population-based study in Western Sweden. Arch Dis Child 89:148–151.

Rauch A, Pfeiffer RA, Leipold G, Singer H, Tigges M, Hofbeck M. 1999. A novel 22q11.2 microdeletion in DiGeorge syndrome. Am J Hum Genet 64:659–666.

Rauch A, Zink S, Zweier C, Thiel CT, Koch A, Rauch R, Lascorz J, Huffmeier U, Weyand M, Singer H, Hofbeck M. 2005. Systematic assessment of atypical deletions reveals genotype-phenotype correlation in 22q11.2. J Med Genet 42:871–876.

Rodningen OK, Prescott T, Eriksson AS, Rosby O. 2008. 1.4Mb recurrent 22q11.2 distal deletion syndrome, two new cases expand the phenotype. Eur J Med Genet 51:646–650.

Ryan AK, Goodship JA, Wilson DI, Philip N, Levy A, Seidel H, Schuffenhauer S, Oechsler H, Belohradsky B, Prieur M, Aurias A, Raymond FL, Clayton-Smith J, Hatchwell E, McKeown C, Beemer FA, Dallapiccola B, Novelli G, Hurst JA, Ignatius J, Green AJ, Winter RM, Brueton L, Brondum-Nielsen K, Scambler PJ. 1997. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: A European collaborative study. J Med Genet 34:798–804.

Saitta SC, Harris SE, Gaeth AP, Driscoll DA, McDonald-McGinn DM, Maisenbacher MK, Yersak JM, Chakraborty PK, Hacker AM, Zackai EH, Ashley T, Emanuel BS. 2004. Aberrant interchromosomal exchanges are the predominant cause of the 22q11.2 deletion. Hum Mol Genet 13: 417–428.

Sanna-Cherchi S, Kiryluk K, Burgess KE, Bodria M, Sampson MG, Hadley D, Nees SN, Verbitsky M, Perry BJ, Sterken R, Lozanovski VJ, Materna-Kiryluk A, Barlassina C, Kini A, Corbani V, Carrea A, Somenzi D, Murtas C, Ristoska-Bojkovska N, Izzi C, Bianco B, Zaniew M, Flogelova H, Weng PL, Kacak N, Giberti S, Gigante M, Arapovic A, Drnasin K, Caridi G, Curioni S, Allegri F, Ammenti A, Ferretti S, Goj V, Bernardo L, Jobanputra V, Chung WK, Lifton RP, Sanders S, State M, Clark LN, Saraga M, Padmanabhan S, Dominiczak AF, Foroud T, Gesualdo L, Gucev Z, Allegri L, Latos-Bielenska A, Cusi D, Scolari F, Tasic V, Hakonarson H, Ghiggeri GM, Gharavi AG. 2012. Copy-number disorders are a common cause of congenital kidney malformations. Am J Hum Genet 91:987–997.

Shaikh TH, O'Connor RJ, Pierpont ME, McGrath J, Hacker AM, Nimmakayalu M, Geiger E, Emanuel BS, Saitta SC. 2007. Low copy repeats mediate distal chromosome 22q11.2 deletions: Sequence analysis predicts breakpoint mechanisms. Genome Res 17:482–491.

Stewart TL, Irons MB, Cowan JM, Bianchi DW. 1999. Increased incidence of renal anomalies in patients with chromosome 22q11 microdeletion. Teratology 59:20–22.

Tan TY, Collins A, James PA, McGillivray G, Stark Z, Gordon CT, Leventer RJ, Pope K, Forbes R, Crolla JA, Ganesamoorthy D, Burgess T, Bruno DL, Slater HR, Farlie PG, Amor DJ. 2011. Phenotypic variability of distal 22q11.2 copy number abnormalities. Am J Med Genet A 155A: 1623–1633.

Vallejo-Illarramendi A, Zang K, Reichardt LF. 2009. Focal adhesion kinase is required for neural crest cell morphogenesis during mouse cardiovascular development. J Clin Invest 119:2218–2230.

Verhagen JM, Diderich KE, Oudesluijs G, Mancini GM, Eggink AJ, Verkleij-Hagoort AC, Groenenberg IA, Willems PJ, du Plessis FA, de Man SA, Srebniak MI, van Opstal D, Hulsman LO, van Zutven LJ, Wessels MW. 2012. Phenotypic variability of atypical 22q11.2 deletions not including TBX1. Am J Med Genet A 158A:2412–2420.

Wu HY, Rusnack SL, Bellah RD, Plachter N, McDonald-McGinn DM, Zackai EH, Canning DA. 2002. Genitourinary malformations in chromosome 22q11.2 deletion. J Urol 168:2564–2565.

Yu S, Graf WD, Ramalingam A, Brawner SJ, Joyce JM, Fiedler S, Zhou XG, Liu HY. 2011. Identification of copy number variants on human chromosome 22 in patients with a variety of clinical findings. Cytogenet Genome Res 134:260–268.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.at the publisher's web-site.