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Microduplication 22q11.2, an Emerging Syndrome: Clinical, Cytogenetic, and Molecular Analysis of Thirteen Patients

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Chromosome 22, particularly band 22q11.2, is predisposed to rearrangements due to misalignments of low-copy repeats (LCRs). DiGeorge/velocardiofacial syndrome (DG/VCFS) is a common disorder resulting from microdeletion within the same band. Although both deletion and duplication are expected to occur in equal proportions as reciprocal events caused by LCR-mediated rearrangements, very few microduplications have been identified. We have identified 13 cases of microduplication 22q11.2, primarily by interphase fluorescence in situ hybridization (FISH). The size of the duplications, determined by FISH probes from bacterial artificial chromosomes and P_1 artificial chromosomes, range from 3–4 Mb to 6 Mb, and the exchange points seem to involve an LCR. Molecular analysis based on 15 short tandem repeats confirmed the size of the duplications and indicated that at least 1 of 15 loci has three alleles present. The patients' phenotypes ranged from mild to severe, sharing a tendency for velopharyngeal insufficiency with DG/VCFS but having other distinctive characteristics, as well. Although the present series of patients was ascertained because of some overlapping features with DG/VCF syndromes, the microduplication of 22q11.2 appears to be a new syndrome.

Introduction

Susceptibility of the chromosome 22q11 region to rearrangements has been recognized on the basis of common clinical disorders such as DiGeorge/velocardiofacial syndrome (DG/VCFS [MIM 188400 and MIM 192430]) and cat-eye syndrome (MIM 115470), which are associated with either decreased or increased gene dosage (McDermid and Morrow 2002). The 22q11.2 microdeletion syndrome, DG/VCFS, is the most common of these conditions, representing a spectrum of clinical anomalies affecting multiple organ systems including cardiovascular, neurological, psychiatric, endocrinologic, and immunologic. Palatal abnormalities and characteristic facial features also can be present.

Recent evidence has implicated low-copy repeats (LCRs) on 22q as mediators of nonallelic homologous recombination (NAHR) that result in rearrangements of 22q (Stankiewicz and Lupski 2002*a*). In theory, both deletion and duplication events should occur in equal proportions, as a result of NAHR caused by unequal crossovers of LCRs. Surprisingly, carriers of duplication of 22q11.2 are rarely reported (Taylor et al. 1977; Reiss et al. 1985; Knoll et al. 1995; Lindsay et al. 1995; Prasher et al. 1995; Fujimoto and Lin 1996; Meins et al. 2003). Only one patient has been described with a "microduplication" of this region: a 4-year-old child with mild dysmorphism, developmental delay, and velopharyngeal insufficiency (Edelmann et al. 1999*b*). Two other patients have been described briefly in abstracts (Hassed et al. 2002; Papenhausen et al. 2002).

Here, we report the phenotypic, cytogenetic, and molecular findings from 13 patients with new diagnoses of variable microduplications of the 22q11.2 region, defining a new chromosomal microduplication syndrome. The duplications range from ~3 Mb (most common) to ~4 Mb and ~6 Mb within the 22q11.21-11.23 band.

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Material and Methods

Clinical Evaluation

The phenotypic presentation of all reported index patients (tables 1 and 2) prompted clinical diagnostic con-

Table 1

Results of the Cytogenetic and Molecular Cytogenetic Studies and Clinical Characterization of 13 Patients with 22q11.2 Microduplications

	Patient													
	1	2	3	4	5[a]	6	7[b]	8	8M[c]	8S1[d]	8S2[e]	9	10[b]	Total
Size of 22q11.2 Duplication (in Mb)	~3	~3	~3	~3	~3	~3	~3	~4	~4	~4	~4	~6	~6	
Karyotype (band stage)	46,XY[f] (550)	46,XY,dup(22) (675)	46,XY,?dup(22) (600)	46,XY[f] (525)	46,XY[f] (450)	46,XY,?dup(22) (550)	NA	46,XY[f] (475)	NA	NA	NA	46,XX,dup(22) (550)	46,XX (NA)	
Parental studies	−	ND	ND	−	−	ND	ND	+	−	+	+	+[g]	−	
Age at diagnosis	3 wk	18 y	13 d	6 y	5 d	5 y	6 mo	13 y	31 y	8 y	10 y	8 y	8 y	
Sex	M	M	M	M	M	M	F	M	F	F	F	F	F	
Heart defect	+[h]	−	−	−	+[i]	−	?	−	−	−	−	−	−	2/12
Velopharyngeal insufficiency	+	?[j]	+	+	?	+	+	−	?	+	−	−	+	7/10
With cleft palate	+	−	+	−	−	+	+	−	−	+	−	−	−	5/13
Hearing loss	+	+	+	−	−	+	?	−	−	−	−	+	−	5/12
Urogenital anomalies	−	−	−	+[k]	+[l]	?	?	+[m]	+[n]	−	−	+[o]	−	5/11
Absent thymus, T cell deficiency	−	−	−	−	+	−	?	−	−	−	−	−	−	1/12
Asplenia	−	−	−	−	+	−	?	−	−	−	−	−	−	1/12
Other malformations/anomalies	−	+[p]	+[q]	+[r]	−	+[s]	?	+[t]	−	+[u]	−	+[v]	−	7/12
Dysmorphic facial features (table 2)	+	+	+	+	+	+	?	+	+	+	+	+	+	12/12
Poor growth (weight and/or length ⩽10th percentile)	−	+	+	−	+	+	?	+	−	−	+	+	−	7/12
Cognitive deficits	Mild	Severe	Mild-moderate	Mild-moderate	?	Moderate	?	Moderate	LD	LD	LD	Mild-moderate	LD	11/11
With motor delays	+	+	+	+	?	+	?	+	?	−	?	+	−	7/9
Poor coordination	−	+	−	+	−	+	?	+	−	−	−	−	−	4/12
Tone abnormality	−	−	↑	↓	−	?	?	−	−	−	−	−	−	2/11
Aggressive behavior	?	+	?	−	?	−	?	+	−	−	−	−	−	2/9
Depression/anxiety	?	−	?	+	?	−	?	+?	+	−	−	−	−	3/9
ADD	?	−	?	−	?	+	?	−	−	−	−	−	+	2/9
Abnormal EEG	−	+	+	−	+	−	?	−	−	−	−	?	−	3/11
With seizures	−	+	−	−	−	−	?	−	−	−	−	+?	−	2/12

Note.—NA = not available; ND = not done; y = years; ? = unknown; LD = learning disabilities; ↑ = hypertonia; ↓ = hypotonia; +? = questionable.

[a] Death at age 1 mo, secondary to cardiac complications. Because of immunodeficiency and recurrent sepsis, the patient was not considered a suitable candidate for surgical procedures at his local institutions.

[b] Limited information available.

[c] Mother of patient 8.

[d] Sibling of patient 8.

[e] Sibling of patient 8.

[f] The duplication was detectable by banded chromosome analysis at the 750–800-band resolution.

[g] The patient's mother has learning disabilities and hearing loss.

[h] Fallot tetralogy.

[i] Hypoplastic left heart syndrome and interrupted aortic arch.

[j] Oral cavity not examined, because patient kept jaws clenched.

[k] Urethral stenosis.

[l] Hypospadias grade I and mild left hydronephrosis.

[m] Grade I right hydronephrosis.

[n] Kidney anomaly requiring surgery at age 8 years.

[o] Urethral stenosis.

[p] Strabismus.

[q] Contractures of the metacarpophalangeal joints of all fingers bilaterally; three subcutaneous cysts at chest, abdomen, and submandibular region; and sacral pit.

[r] Hyperopia.

[s] Ankyloglossia.

[t] Possible proximal radioulnar synostosis, hyperopia, accelerated puberty, and headaches.

[u] Hyperopia.

[v] Intestinal malrotation/volvulus and hyperopia/astigmatism.

Table 2

Dysmorphic Features Of Patients with 22q11.2 Microduplications

Dysmorphic Feature	Patient 1	2	3	4	5[a]	6	8	8M	8S1	8S2	9	10	Edelmann et al. (1999*b*)	Total
Head:														
Long narrow face	−	−	+	+	+	−	+	−	−	+	−	−	+	6/13
Bitemporal narrowing	−	−	+	+	?	−	−	−	−	−	+	−	−	3/12
Microcephaly	−	+	−	−	−	−	+	−	−	−	−	−	−	2/13
Brachycephaly	+	−	−	−	−	?	−	−	−	−	+	−	−	2/12
Mild micro-/retrognathia	+	−	+	+	?	−	−	+	+	+	−	−	−	6/12
Eyes:														
Superior placement of eyebrows	−	+	+	+	+	+	+	−	−	−	+	+	+	***9/13***
Ptosis	−	−	−	+	?	−	+	−	−	+	−	−	−	3/12
Epicanthal folds	+	−	+	−	?	−	−	−	−	−	−	−	−	2/12
Appearance of widely spaced eyes	−	+	+	+	+	+	−	+	+	−	+	+	−	***9/13***
Palpebral fissures:					?									
Upslanting	+		+				−							2/12
Downslanting		+		+		+	−	+	+	+	+	+	+	***9/12***
Ears:														
Minor ear malformations	−	+[b]	+[c]	+[b]	−	+[d]	?	+[e]	−	−	−	−	−	5/12
Nose:														
Large nose	−	+[f]	−	−	+[f]	+[f]	−	−	−	−	+[g]	−	−	4/13
Hands/feet:														
Abnormal palmar creases	+[h]	−	+[i]	+[j]	−	−	−	−	−	−	−	−	−	3/13
Long fingers and/or toes	−	+	+	−	?	−	−	−	−	−	−	−	−	2/12
Brachydactyly	−	−	−	−	?	−	+	+	+	−	−	−	−	3/12

Note.—Boldface italic type indicates that >50% of patients had the feature. No information on facial features was available for patient 7.

[a] Patient died at age 1 mo.

[b] Simplified ears.

[c] Slightly posteriorly rotated.

[d] Preauricular tag on the left; pit on the posterior right lobule.

[e] Prominent antihelix.

[f] Broad tip.

[g] Long and squared.

[h] Deep palmar creases.

[i] Not specified.

[j] Unilateral bridged palmar crease.

sideration of DG/VCFS. Patients 1, 2, 3, 4, 5, 6, and 8, as well as the mother and two sisters of patient 8 (fig. 1), were directly examined by at least one of the authors (R.E., N.L., V.M., A.A.A., W.S., E.S.F., J.G., R.C., and M.M.). Clinical information on patient 9 (fig. 1) was collected by review of medical records and by interview of the patient's primary care physician, after appropriate consent was obtained from the patient's parents. Limited information was available on patients 7 and 10. The patients' parents gave written informed consent for publication of photographs. Mayo Clinic institutional review board approval was obtained for extended laboratory studies and publication.

Cytogenetic Analysis

Blood samples from 653 consecutive patients referred to rule out DG/VCFS were processed by standard chromosome and/or FISH procedures. At least 20 GTL-banded (G bands by Giemsa, using Leishman's stain) metaphase cells were analyzed for each patient (fig. 2). FISH was performed using DNA fluorescent probes for the DG/VCFS critical region (TUPLE1 [MIM 600237]) at 22q11.2 and a control probe, arylsulfatase-A (ARSA [MIM 607574]), at 22q13.3, from a commercially available source (Vysis). This is a direct-labeled dual-color probe mixture with TUPLE1 (HIRA) probe labeled in orange and ARSA probe labeled in green. Slide preparation, denaturation, hybridization, and posthybridization washes were all performed according to already established procedures (Crifasi et al. 1995) and the manufacturer's recommendations, with minor modifications. For each patient, at least 100 interphase and 10 metaphase cells were scored for both the TUPLE1 and ARSA signals. Those cases that did not have TUPLE1 deletion were processed for microduplication of 22q11.2.

To establish a normal cutoff value for duplication in-

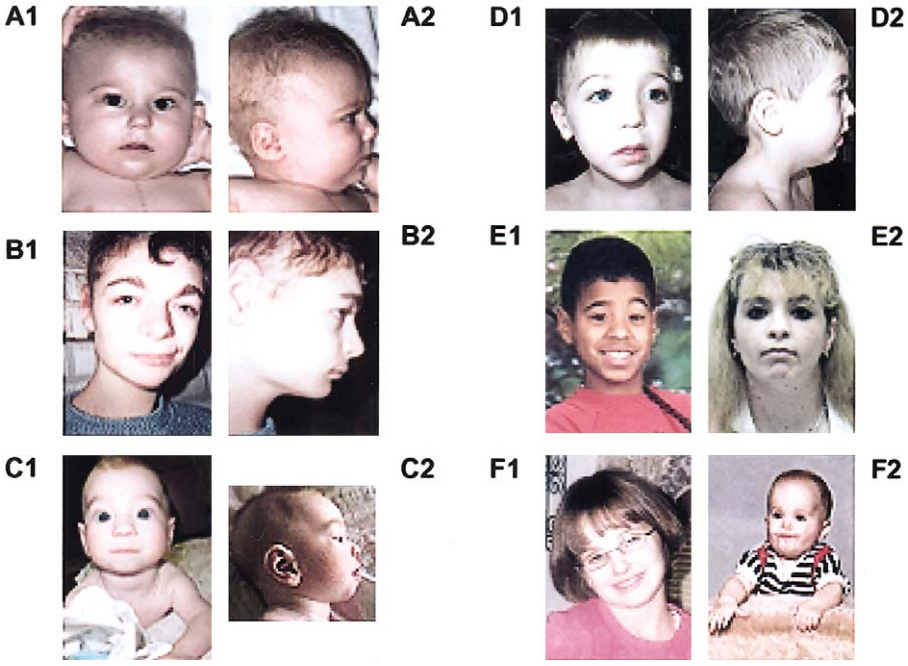

Figure 1 *A–F,* Photographs of seven patients with 22q11.2 microduplications. For description of the patients' main dysmorphic features, see table 2. Additional dysmorphic stigmata of each patient are outlined here. *A1* and *A2,* Patient 1 at age 11 mo, with flat occiput, long eyelashes, medial eyebrow flaring, long philtrum, and a thin upper lip. Not shown: short frenulum of the tongue, narrow ear canals, persistent fingertip fat pads, and the vertical plantar crease between the first and second toes, present bilaterally. *B1* and *B2,* Patient 2 at age 18 years, with a relatively large mouth, posteriorly sloping forehead, slightly overfolded ears, thick eyebrows, and alopecia due to an X-linked hypotrichosis syndrome, which was also present in his mentally normal brothers. *C1* and *C2,* Patient 3 at age 7 mo. Not shown: protruding tongue and fifth-finger clinodactyly. *D1* and *D2,* Patient 4 at age 6 years. Not shown: extremely high arched palate, three café-au-lait macules (<0.5 cm). *E1,* Patient 8 at age 10 years. He had surgery for left ptosis at age 5 years. *E2,* Mother of patient 8, at age 31 years. *F1,* Patient 9 at age 12 years. *F2,* Patient 9 at age 9.5 mo. Not shown: distal placement of the thumb with decreased abduction.

volving TUPLE1, the probe was hybridized to interphase cells from 10 unaffected individuals, and 200 cells were scored for both the TUPLE1 and ARSA signals in each of these samples. Strict scoring criteria were applied during the analysis of interphase cells, to differentiate between fragmented/split probe signals and duplicated critical region probe signals, thereby eliminating false positives. Duplication was ruled out if split TUPLE1 signals were present with split ARSA signals, if split TUPLE1 signals were not equal in size and intensity with the third TUPLE1 signal, or if split TUPLE1 signals had a visible connecting strand. On the other hand, if the split TUPLE1 signals were equal in size and intensity with the third TUPLE1 signal and if a signal of equal size could fit in-between, the cell was scored as a duplication (fig. 3). On the basis of the highest proportion of abnormal signal, a normal cutoff of 11.5% was established at a 95% CI from a one-sided binomial distribution. A sample with duplication therefore needs to have >11.5% nuclei with three TUPLE1 signals. Ten metaphases (fig. 3) were examined to confirm the interphase finding in each case, but duplication in the metaphase nuclei is very difficult to detect; therefore, the primary criteria used for diagnosis of duplication were based on the analysis of interphase FISH (table 3). When possible, family members of patients with the 22q11.2 duplication were also analyzed.

Molecular Cytogenetic Analysis

To estimate the size of the 22q11.2 duplications in each patient, 25 direct-labeled FISH probes (20 were informative) were designed from BACs and PACs spanning regions centromeric and telomeric to TUPLE1. The search for BACs and PACs in the region of interest on 22q11.2 was accomplished using the University of California Santa Cruz (UCSC) Genome Browser (UCSC Genome Bioinformatics Home Page). Clones were selected on the basis of their location, such that each clone flanked or overlapped the next clone. Stab cultures of the BAC and PAC clones were received from Dr. B. Roe of the Department of Chemistry and Biochemistry, University of Oklahoma. Clones were plated and propagated immediately upon arrival, and glycerol stocks were prepared. Isolation and purification of DNA were performed, using the QIAGEN Plasmid Maxi Kit (25), ac-

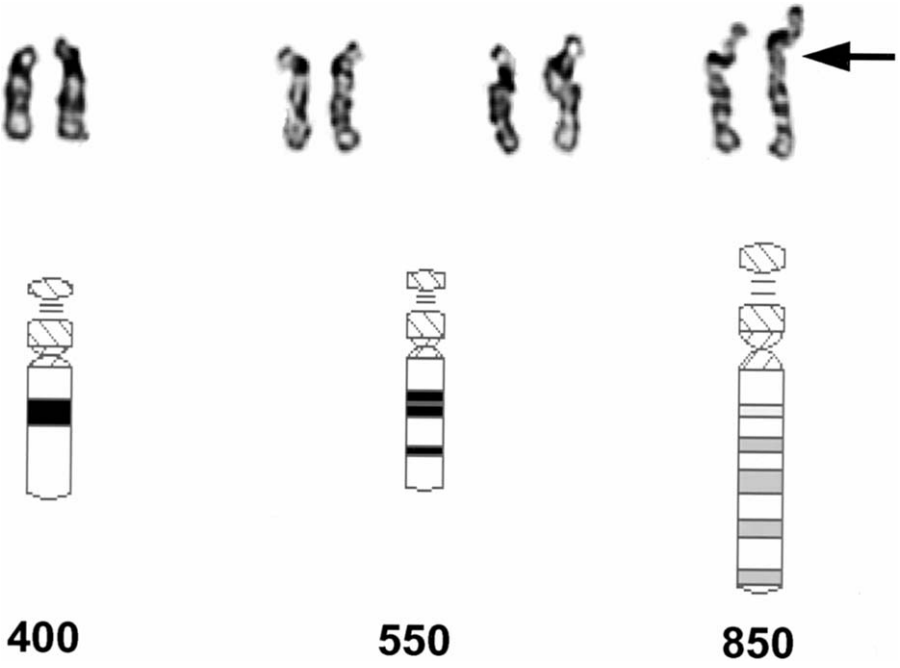

Figure 2 G-banded chromosome 22 pair from patient 8, at different band resolutions. The chromosome with dup(22)(q11.2) is to the right in each pair. The dup(22)(q11.2) is readily visible at the 850-band stage (*arrow*).

cording to manufacturer's instructions. The sequences for the BACs and PACs were obtained from the National Center for Biotechnology Information (NCBI) Web site. After editing out repetitive sequences through use of the CENSOR server (Genetic Information Research Institute Web site), primers were designed for each BAC and PAC clone, and unique sequences were amplified by PCR to verify the identity of the clone.

Nick translation was performed using the Vysis Nick Translation Kit (Vysis) to produce the fluorescence-labeled DNA probes for FISH. Probes were labeled with Spectrum Orange-dUTP or Spectrum Green-dUTP, precipitated, and applied to metaphase cells from normal blood specimens, to verify hybridization to the region on 22q and the lack of hybridization to other sequences. The validated probes were then sequentially applied to interphase and metaphase cells from the 13 patients, as described for the TUPLE1 probe above. The status of each probe was determined from the analysis of signals from 100 interphase cells and 10 metaphase cells from each of the patients.

Molecular Analysis: Polymorphism Analysis Using STR Markers

Genomic DNA was extracted, from peripheral white blood cells that were either freshly obtained or fixed in methanol/acetic acid, through use of either the Qiagen protocol (Qiagen) or the Puregene protocol (Gentra Systems). Polymorphism data were collected using a panel of 15 STR fluorescent markers (D22S420, D22S427, D22S1638, D22S941, D22S1648, D22S944, D22S1623, D22S264, D22S311, D22S1709, D22S308, D22S306, D22S425, D22S303, and D22S257). All of the primer sequences for the polymorphic markers were obtained from The Genome Database, except for D22S420, which is from the ABI PRISM Linkage Mapping Set (Applied Biosystems). The forward primer for each marker was labeled with either 6-FAM or NED, and PCR was performed using conditions optimized for each marker. The amplified products were diluted with water, to obtain a fluorescent signal strength of 1,000–6,000 relative fluorescence units, and were analyzed on an ABI PRISM 3100 using Genotyper 3.7 software (Applied Biosystems). Duplication was noted whenever three distinct alleles were present. If only two alleles were present, dosage analysis was performed to determine whether duplication was present, by comparing the ratio of the peak areas with the ratio obtained from known control samples (Krepischi-Santos and Vianna-Morgante 2003).

Results

Over a 1-year period, blood samples from 653 consecutive patients who were referred to rule out DG/VCFS were processed for standard chromosome and/or FISH analysis, to detect deletion or duplication of 22q11.2.

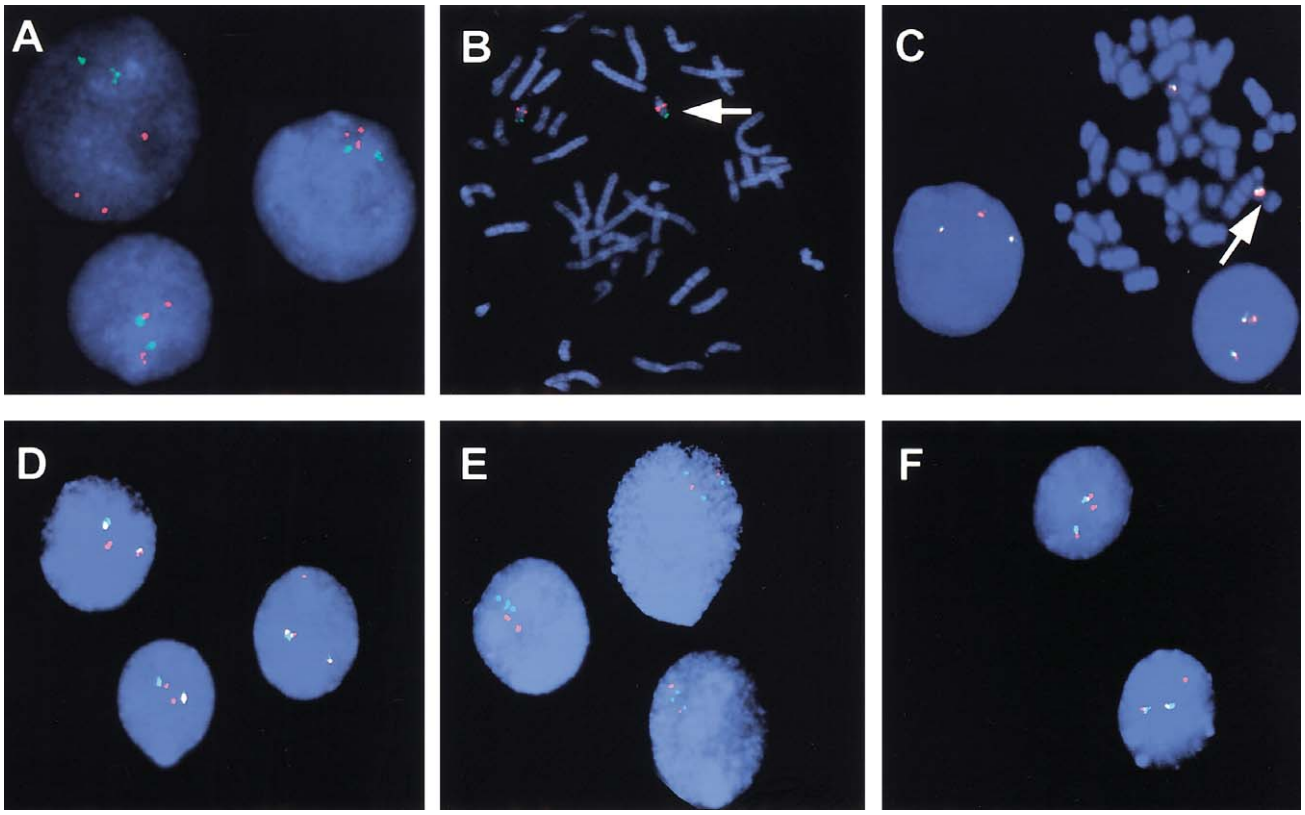

Figure 3 *A,* Interphase cell from patient 1, showing duplication of TUPLE1 (*three red signals*) and two control probe (ARSA) signals (*green*). *B,* Metaphase cell from patient 1, showing microduplication of TUPLE1 (*red*) on one chromosome 22 (*arrow*) and normal control probe, ARSA (*green*). The microduplication is seen as a larger-sized signal compared with the normal, although the distinction was often difficult, underscoring the importance of interphase FISH analysis. *C,* FISH with probes prepared from BACs centromeric to HIRA. Interphase cells from patient 2 are shown, showing two signals for b476c20 (*green*) (base pair positions 16796870–16971860) and three signals (duplication) for bac519d21 (*red*) (base pair positions 16926349–17071935). A metaphase cell shows the same duplication on one chromosome 22 (*arrow*). Overlap of the green and red signals is seen as a yellow signal. Bac519d21 marks the proximal breakpoint of the 22q11 duplication in all 13 patients. *D, E,* and *F,* FISH with probes prepared from clones telomeric to HIRA. *D,* Interphase cells from patient 7 with a ~3-Mb duplication, showing duplication of b135h6 (*red*) (base pair positions 19443166–19678560) and normal signal pattern for RP11-36N5 (*green*) (base pair positions 20289389–20343913). Overlap of the red and green signals is seen as a yellow signal. *E,* Interphase cells from patient 8 with a ~4-Mb duplication, showing duplication of CTA-526G4 (*green*) (base pair positions 20712617–20712739) and normal signal pattern for 865e9 (*red*) (base pair positions 21687068–21830046). *F,* Interphase cells from patient 10 with a ~6-Mb duplication, showing duplication of RP11-76E8 (*red*) (base pair positions 22813079–22989794) and normal signal pattern for RP3-370D16 (*green*) (base pair positions 23137821–23137941).

Samples were initially screened for deletion of 22q11.2. A total of 13 patients (2%) were identified with microduplication. The clinical findings for the patients are presented in tables 1, 2, and 4. The details of laboratory findings corresponding to each case are summarized in tables 1, 3, 5, and 6. Age at diagnosis ranged from newborn to 18 years of age, with 54% of patients being male. Microduplication 22q11.2 was de novo in five of seven cases.

Phenotype

The distinctive facial characteristics observed in more than half of the patients were superior placement of eyebrows and widely spaced eyes with downslanting palpebral fissures (table 2). Mild micro-/retrognathia and minor ear anomalies were seen in 50% and 45% of patients, respectively. Forty-six percent of patients had a long, narrow face. Velopharyngeal incompetence was present in 70% (7/10) of patients, with cleft palate in five subjects (table 1). Seventeen percent (2/12) of patients had congenital heart defects; one of these two patients had Fallot tetralogy, and the other had hypoplastic left heart syndrome and interrupted aortic arch. Forty-two percent (5/12) of patients had hearing loss; one of these five patients had confirmed conductive hearing loss, whereas the exact type of hearing loss was not available in the other cases. Malformations of the urogenital tract were diagnosed in 46% (5/11) of patients; two of these patients had urethral stenosis. Twenty-five

Table 3

Fluorescence In Situ Hybridization of 13 Patients with Microduplication of 22q11.2

Patient	No. of Interphase Cells with dup(22)(q11.2q11.2)/ Total Cells	No. of Metaphase Cells with dup(22)(q11.2q11.2)/ Total Cells
1	87/100	11/11
2	84/100	4/10
3	87/100	10/10
4	85/100	13/13
5	83/100	20/50
6	98/100	8/11
7	70/100	7/11
8	70/100	10/10
8M	155/200	0/10
8S1	87/100	0/10
8S2	81/100	0/10
9	161/200	10/10
10	81/100	4/10

percent of patients had other significant malformations, including absent thymus, asplenia, and intestinal malrotation. Fifty-eight percent of patients had low weight or poor growth, and one required a feeding tube. Cognitive impairment was quite variable, but all patients that could be evaluated had cognitive deficits of some degree. One patient had a seizure disorder, although three more had definitely or possibly abnormal EEG results.

The following two cases exemplify the widely variable phenotype of microduplication 22q11.2 syndrome. Patient 5 (tables 1 and 2) had the most severe clinical presentation. At birth, he received a diagnosis of hypoplastic left heart syndrome and interrupted aortic arch. Additional malformations included absent thymus, asplenia, mild left hydronephrosis, and grade I hypospadias. Because of immunodeficiency and recurrent episodes of sepsis, he was not considered a suitable candidate for surgical procedures at his local institutions. He died of cardiac complications at 1 mo of age. In contrast, patient 8, with familial microduplication 22q11.2 (tables 1 and 2), showed a quite different phenotype, including mental retardation, microcephaly, and aggressive behavior. Except for ptosis and mild hydronephrosis, he had no malformations. There was considerable intrafamilial variability of the condition. The two affected sisters had learning disabilities. In addition, one had a cleft palate and the other had poor growth. The affected mother also had learning problems. She had a kidney anomaly requiring surgery in childhood.

Cytogenetic Findings

Microduplication of 22q11.2 was observed in 13 patients, 10 of whom were unrelated and 3 of whom were related to 1 of the 10 (a mother and two siblings; see tables 1, 2, and 3). Microduplication of 22q11.2 was observed or suspected in four of nine (one was not avail-

Table 4

Comparison of 22q11.2 Deletion and Duplication Disorders

Symptom	% (Fraction) with Symptom in			
	Microduplication 22q11.2	Large Interstitial Duplication 22q11[a]	Cat-Eye Syndrome[b]	Microdeletion 22q11.2[c]
Cognitive deficits	100 (11/11)	100 (7/7)	56 (38/68)	90–100
Downslanting palpebral fissures	75 (9/12)	71 (5/7)	69 (48/70)	Not characteristic
Urogenital malformations	46 (5/11)	57 (4/7)	71 (55/77)	37 (25/67)
Hearing loss	42 (5/12)	33 (1/3)[d]	16 (11/68)	39 (19/49)
Cleft palate or absent uvula	39 (5/13)	0 (0/7)	31 (15/48)	32 (75/234)
Conotruncal heart defects[e]	17 (2/12)[f]	0 (0/7)	9 (7/80)	38 (116/305)
Other heart defects	8 (1/12)[f]	50 (3/6)[d]	88 (70/80)	37 (113/305)
Preauricular malformations	8 (1/12)	71 (5/7)	87 (78/90)	Not characteristic
Immunodeficiency	8 (1/12)	Not characteristic	Not characteristic	77 (46/60)
Anorectal malformations	0 (0/13)	14 (1/7)	81 (71/88)	Rarely reported[g]
Ocular coloboma	0 (0/13)	43 (3/7)	61 (54/88)	Rarely reported[h]
Hypocalcemia	Not detected	Not detected	Not detected	49 (77/158)

[a] Data are from Taylor et al. (1977), Reiss et al. (1985), Knoll et al. (1995), Lindsay et al. (1995), Prasher et al. (1995), Fujimoto and Lin (1996), and Meins et al. (2003). Duplication sizes ranged from 22q11.1-q11.2 to 22q11-q13.

[b] Data are from Rosias et al. (2001).

[c] Data are from Emanuel et al. (2001).

[d] Information was not available in all cases.

[e] At least one of each of the following: tetralogy of Fallot, interrupted aortic arch, and truncus arteriosus.

[f] One patient had hypoplastic left heart syndrome and interrupted aortic arch.

[g] From Worthington et al. (1997).

[h] From Goldberg et al. (1993).

Table 5

Individual FISH Results of Probes from Clones on 22q11.2 for 13 Patients

Clone (GenBank Accession Number)	Base Pair Position[a]	Chromosome Band	FISH Results in Patient[b]												
			1	2	3	4	5	6	7	8	8M	8S1	8S2	9	10
b476c20 (AC016027)	16796870–16971860	22q11.21	+	+	+	+	+	+	+	+	+	+	+	+	+
b677f7 (AC008101)	16893710–17021443	22q11.21	+	+	+	+	+	+	+	+	+	+	+	+	+
bac519d21 (AC008079)	16926349–17071935	22q11.21	++	++	++	++	++	++	++	++	++	++	++	++	++
pac995o6 (AC008132)	17071632–17217229	22q11.21	++	++	++	++	++	++	++	++	++	++	++	++	++
p423 (AC007326)	17263867–17366764	22q11.21	++	++	++	++	++	++	++	++	++	++	++	++	++
72f8 (AC000085)	17621007–17721158	22q11.21	++	++	++	++	++	++	++	++	++	++	++	++	++
TUPLE1 (HIRA)	17692778–17793801	22q11.21	++	++	++	++	++	++	++	++	++	++	++	++	++
p888c9 (AC005663)	18308259–18411380	22q11.21	++	++	++	++	++	++	++	++	++	++	++	++	++
b444p24 (AC007663)	18515763–18684001	22q11.21	++	++	++	++	++	++	++	++	++	++	++	++	++
562f10 (AC007731)	19028370–19204493	22q11.21	++	++	++	++	++	++	++	++	++	++	++	++	++
bac32 (AC007050)	19230748–19394655	22q11.21	++	++	++	++	++	++	++	++	++	++	++	++	++
b135h6 (AC002470)	19443166–19678560	22q11.21	++	++	++	++	++	++	++	++	++	++	++	++	++
RP11–36N5	20289398–20343913	22q11.21	+	+	+	+	+	+	+	NT	NT	NT	NT	NT	NT
RP11–22M5	20564423–20761060	22q11.22	+	+	+	+	+	+	+	NT	NT	NT	NT	NT	NT
CTA-526G4	20712617–20712739	22q11.22	+	+	+	+	+	+	+	++	++	++	++	++	++
865e9 (AC000029)	21687068–21830046	22q11.22	+	+	+	+	+	+	+	+	+	+	+	++	++
605b (AC000102)	21829574–21833044	22q11.23	+	+	+	+	+	+	+	+	+	+	+	++	++
RP11–76E8	22813079–22989794	22q11.23	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	++	++
RP3–370D16	23137821–23137941	22q11.23	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	++	+
RP5–930L11	23510702–23645658	22q11.23	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	+	+
Estimated duplication size (Mb)			3	3	3	3	3	3	3	4	4	4	4	6	6

[a] NCBI *Homo sapiens* genome view, build 33 (NCBI Map Viewer Web site).

[b] + = normal signal pattern; ++ = duplication; NT = not tested.

able for chromosome analysis) unrelated patients, on the basis of 550–675-band-stage karyotype analysis (table 1). However, at the 750–850-band stage, the duplication was detectable in all eight patients available to us for chromosome analysis (fig. 2). By interphase FISH (fig. 3), the duplication was detected with ease in all 13 patients. However, the detection of the duplication by metaphase FISH was inconsistent, ranging from 0/10 to 13/13 cells analyzed (table 3). The duplication was detectable on longer chromosomes, where they were not seen by routine metaphase FISH analysis. Analysis with FISH probes from BACs and PACs indicated the involvement of the same LCR at the centromeric end, but the telomeric end of the duplication varied, resulting in duplication sizes ranging from ~3 Mb to 6 Mb (table 5; fig. 4).

Molecular Analysis

Results of haplotype analysis using 15 STR markers specific for genomic region 22q11.2 were consistent with microduplication in 12 patients tested (table 6). The proximal breakpoint was similar in all 12 patients and was mapped between D22S427 and D22S1638. The distal breakpoint was between D22S1709 and D22S308 in six patients, between D22S306 and D22S425 in four patients (a mother and three children), and through D22S257 in two other patients. All 12 patients demonstrated at least one marker with three alleles through the duplicated region, with the majority of patients having three to five markers showing three alleles. Markers that demonstrated two alleles were subjected to dosage analysis to determine whether duplication was present (fig. 5). The haplotype analysis of three generations of patient 8 showed that the duplication originated in his maternal grandfather (fig. 6).

Discussion

In this investigation, patients who were referred to rule out 22q11.2 microdeletion diagnostic of DG/VCFS were included if the critical-region microdeletion result of metaphase FISH analysis was normal. This study suggests that microduplications of 22q11.2 are relatively common and probably have been underdiagnosed. Use of interphase FISH analysis was primarily responsible for the diagnosis, since routine chromosome analysis can easily miss the duplication. Theoretically, a frequency similar to that of DG/VCFS, 1:4,000–1:6,000 (Shaffer and Lupski 2000; Botto et al. 2003; Kato et al. 2003), is anticipated. By use of interphase FISH and cytogenetic analysis, 13 patients (10 unrelated) with a microduplication of 22q11.2 were identified within 1 year (13/653, or 2% of referrals for deletion of 22q11.2). Within the same period, microdeletions of 22q11.2 (DG/VCFS) were diagnosed in 40 patients (6.1%).

The clinical phenotype of patients in this series with the "22q11.2 duplication syndrome" appears variable,

Table 6

Microsatellite Analysis of 22q11.2 Microduplication Involving 12 Patients

DNA Marker	Position on UCSC Physical Map (Mb)	Allele(s) in Patient[a]											
		1	2	3	4	5	6	8	8M	8S1	8S2	9	10
D22S420	16.23	2, 3	4, 5	NA	2, 3	2, 4	2, 3	2, 3	2, 2	2, 3	2, 4	2, 3	3, 4
D22S427	16.97	4, 7	3, 4	2, 2	2, 7	6, 7	2, 7	2, 4	2, 4	2, 4	4, 5	2 (NI)	2, 7
D22S1638	17.37	1, 3, 3	2, 3, 6	NA	5 (NI)	2 (NI)	3, 5	3, 3, 4	1, 3, 4	3, 3, 4	2, 3, 4	3, 3, 7	2, 3, 4
D22S941	17.78	2 (NI)	1, 2, 6	NA	2, 2, 5	3, 4, 5	2, 5, 7	2, 2, 3	1, 2, 3	2, 2, 3	1, 2, 3	2, 2, 3	1, 1, 2
D22S1648	17.18	1 (NI)	1 (NI)	NA	1 (NI)	3, 4, 5	1 (NI)	1, 2, 3	1, 1, 3	1, 2, 3	1, 1, 3	1 (NI)	1 (NI)
D22S944	17.98	2, 2, 5	1, 2, 5	1, 1	1, 1, 2	2 (NI)	2, 2, 3	1, 2	2, 3	1, 2	2 (NI)	1, 2, 3	2, 4, 4
D22S1623	18.02	1, 2, 3	2, 2, 3	NA	2, 2, 3	3, 5, 5	2, 3	1, 4, 4	2, 4, 4	1, 4, 4	1, 4, 4	NA	NA
D22S264	19.10	NA	1, 2, 5	1, 2, 7	2, 5, 7	3, 7, 8	3, 7, 8	2, 3, 5	2, 3, 5	2, 3, 5	2, 3, 4	2, 6, 7	2, 2, 6
D22S311	19.50	1, 2, 4	3, 3, 5	NA	1, 3, 5	1, 4, 4	3, 5, 5	1, 2, 3	1, 2, 1	1, 2, 3	1, 2, 3	1, 2, 4	1, 1, 3
D22S1709	19.74	1, 2, 3	5, 5, 6	NA	2, 5, 7	1, 4, 4	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 3	1, 1, 3	4 (NI)	1, 2, 5
D22S308	20.83	3 (NI)	1, 3	NA	1, 3	3 (NI)	1, 3	1, 3, 3	1, 3, 3	1, 3, 3	3 (NI)	1, 3, 3	1, 1, 3
D22S306	20.89	NA	2, 4	1, 2	1 (NI)	2, 3	1, 2	1, 2, 2	2 (NI)	1, 2, 2	1, 2, 2	1, 3, 3	3 (NI)
D22S425	21.41	1, 4	2, 6	NA	1, 7	1, 4	1 (NI)	1, 3	1, 3	1, 3	1, 2	1, 3, 5	2, 2, 4
D22S303	21.60	1, 2	3 (NI)	NA	1, 2	1, 2	2, 3	1, 3	1, 3	1, 3	1, 4	1, 2, 3	1, 2, 2
D22S257	21.89	1, 4	1, 5	NA	4 (NI)	1, 4	1, 4	1, 3	1, 4	1, 3	1, 3	1, 3, 4	1, 4, 4

[a] NI = noninformative; NA = no amplification.

ranging from mild learning disabilities as the only symptom to the presence of severe congenital malformations leading to early death (tables 1 and 2; fig. 1). Multiple organ systems can experience effects; these effects include hearing loss, congenital heart defects, growth deficiency, and global developmental delays. Two patients showed conotruncal defects commonly seen in patients with DG/VCFS (Emanuel et al. 2001). Certain clinical symptoms, such as palatal clefting/insufficiency or thymus aplasia, are features also observed in patients with DG/VCFS. In all 10 unrelated patients, DG/VCFS was clinically suspected, either because of the patient's clinical presentation and/or a family history suggestive of an underlying 22q11.2 microdeletion. Because this series of patients was ascertained as suspected DG/VCFS cases, the group may be biased toward DG/VCFS-like features. A prospective study without this inherent bias could produce different frequencies of associated anomalies.

We suggest that the following features may be associated with the condition: appearance of widely spaced eyes and superior placement of eyebrows—that is, increased distance from eyebrow to upper eyelid crease; downslanting palpebral fissures with or without ptosis; mild micro-/retrognathia; and a long, narrow face. These findings are different from dysmorphism observed in patients with DG/VCFS. Of note, infants might not present with the same phenotypic features as older children (fig. 1). Since the clinical presentation can be mild and facial features can be divergent from those of DG/VCFS, many patients with the disease might be without diagnoses.

It is interesting that one patient presented with preauricular tags and pits, which have been reported to be the most consistent finding in cat-eye syndrome (Rosias et al. 2001). Likewise, most patients with an interstitial microduplication or large duplication of 22q11 presented with downslanting palpebral fissures, which is a frequent feature in cat-eye syndrome but not in 22q11.2 microdeletion syndrome. However, other findings associated with cat-eye syndrome, such as anorectal anomalies and ocular coloboma, were not observed in our series of patients (table 4).

Marked inter- and intrafamilial variability is observed among patients with 22q11.2 microduplications. Different degrees of mental impairment, for example, may be present within the same family despite the family members having the same duplication size. Two of seven of the presented cases in which parents have been tested were familial (table 1). The recurrence risk of 50% for each child of an affected parent may result in multiple affected family members and, thus, make this disorder a significant health concern. Early and precise diagnosis of the condition is imperative to provide adequate genetic counseling.

Chromosome-banding studies at <550-band resolutions may not always reveal microduplication of chromosome 22q11.2, since the microduplication is in a light-banded region. Among the nine patients studied chromosomally, the dup(22)(q11.2) was identified from 550–675-band resolution G-banded karyotypes in two patients and was suspected in two other patients (table 1). The microduplication was, however, visible at higher resolution (750–850-band stage) in all of the studied patients (fig. 2). Since 750–800-band-resolution karyotypes are not routinely analyzed, FISH studies of inter-

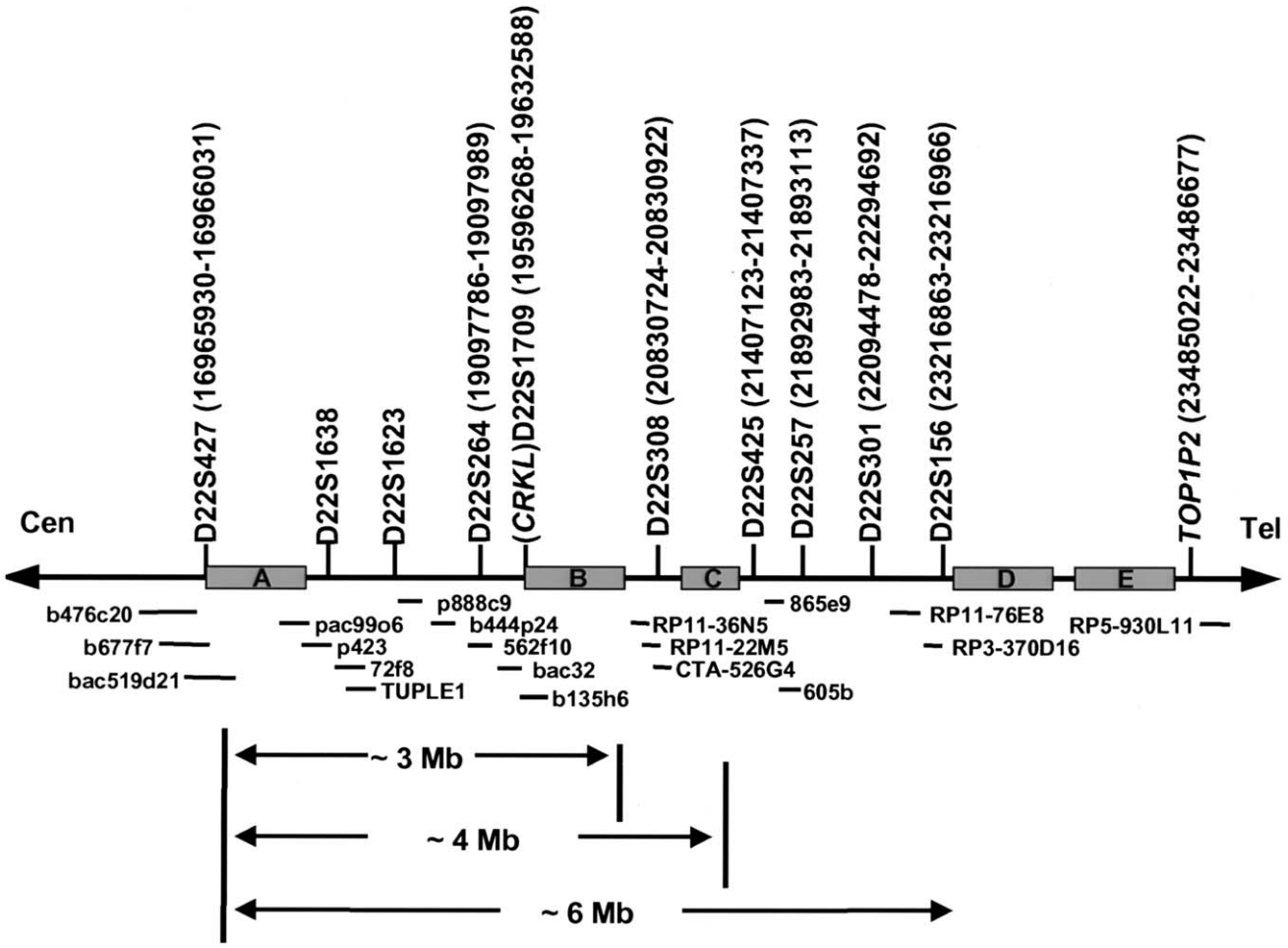

Figure 4 Comparison of the microduplication breakpoints determined by BAC and PAC mapping of 22q in 13 patients with dup(22)(q11.2) with the proximal and distal LCRs (denoted by boxes marked "A" and "B," respectively) of the 3-Mb DG/VCFS deletion. Boxes marked "C," "D," and "E" indicate other documented 22q11.2 LCRs, which are telomeric to the distal LCR (B). BAC519d21 marks the proximal breakpoint of the duplicated segment of 22q11.2 in all 13 patients. The distal breakpoints were between b135h6 and RP11-36N5 for ~3-Mb duplications, between CTA-526G4 and 865e9 for ~4-Mb duplications, and between RP11-76E8 and RP3-370D16 or RP5-930L11 for ~6-Mb duplications. Seven patients had ~3-Mb duplications, whereas four and two had ~4 Mb and ~6 Mb duplications, respectively. Numbers in brackets indicate the base pair positions of the genetic markers. Information used for the figure was obtained from Collins et al. (1997), McTaggart et al. (1998), Edelmann et al. (1999*b*), and NCBI Map Viewer (Build 33, April 2003).

phase nuclei play a key role in the identification of patients with dup(22)(q11.2).

Interphase FISH detected the duplication in all patients, but it was confirmed by metaphase FISH in all but three patients. An average of 90% of interphase cells showed three TUPLE1 signals, whereas the microduplication ranged from 0% to 100% in metaphase cells (table 3). This again underscores the need for high-resolution chromosomes in the identification of microduplications, both for G-banded analysis and metaphase FISH, which may not be practicable in all cases. Recently, it was demonstrated that repetitive DNA sequences—namely, the region-specific LCRs—are present at the breakpoints of common chromosome 22 rearrangements as well as at the breakpoints of common rearrangements involving other chromosomes (Edelmann et al. 1999*a*, 1999*b*; Shaffer and Lupski 2000; Shaikh et al. 2000, 2001; Stankiewicz and Lupski 2002*b*). Since LCRs are involved in the interstitial deletion of 22q11.2 resulting in DG/VCFS, and because of the relative frequencies of this disorder, one would expect that the reciprocal microduplication 22q11.2 would be of similar frequency. The paucity of reported cases of 22q11.2 microduplications likely reflects a limitation in the techniques used to detect microdeletions and/or the mild nature of anomalies in some patients.

In an effort to determine the sizes of duplications in the 13 patients, FISH analyses using BAC and PAC

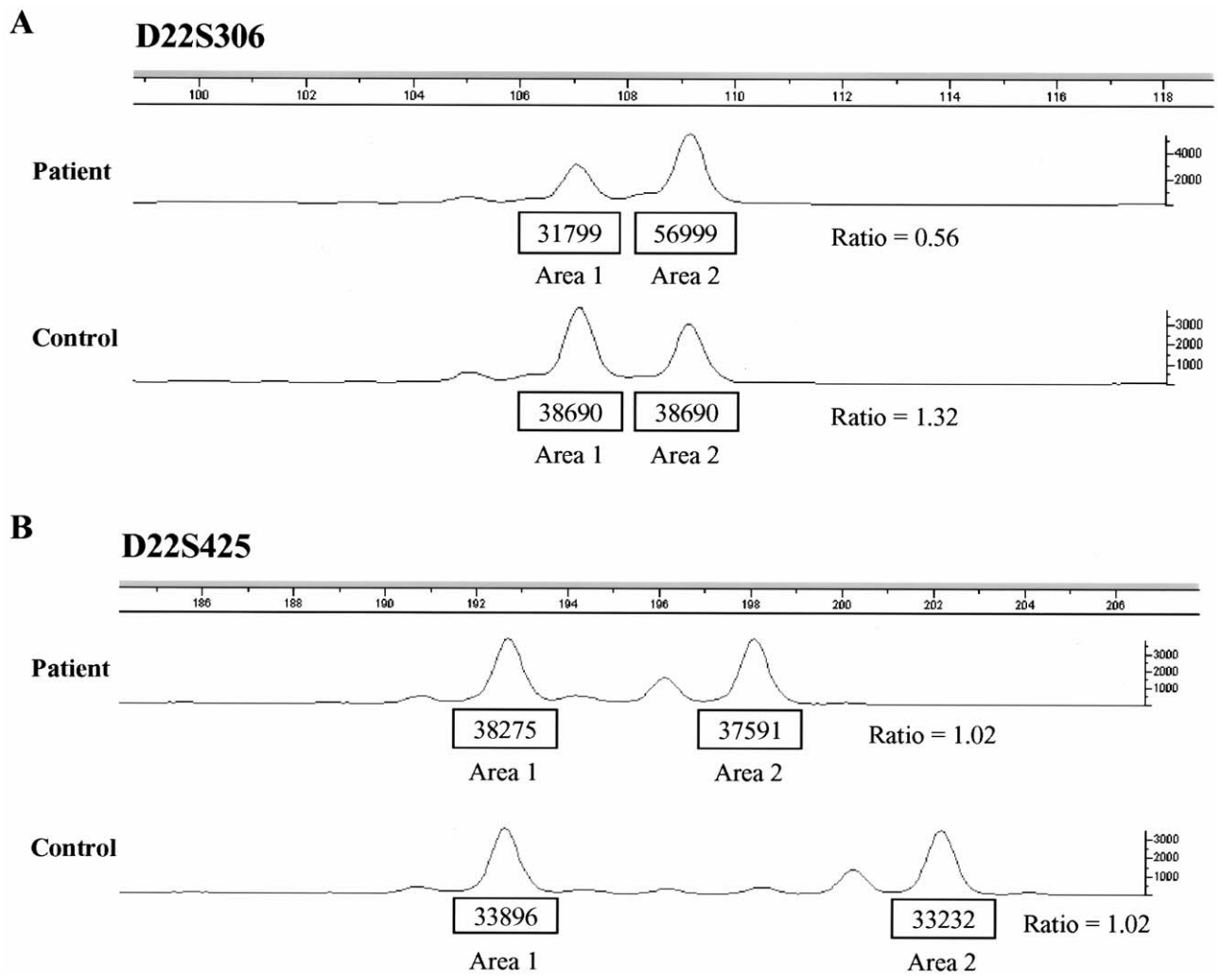

Figure 5 Dosage analysis used to determine duplication size by microsatellite analysis for the family with a ~4-Mb duplication. Ratios (area 1/area 2) were calculated from the areas under each peak for the patient samples and a negative control with the same peak pattern. *A,* Marker D22S306; ratios vary more than twofold, indicating duplication of allele 2. *B,* Marker D22S425; ratios are identical for the patient and the negative control, indicating that no duplication is present. *X*-axis: time in seconds; *Y*-axis: RFUs.

probes on 22q that were centromeric and telomeric to HIRA were performed and showed that all 13 patients had the same centromeric duplication breakpoint. The distal portion of bac519d21 delineates this centromeric breakpoint and maps to the proximal LCR implicated in the 3-Mb DG/VCFS deletion (McTaggart et al. 1998; Edelmann et al. 1999*a,* 1999*b;* Shaffer and Lupski 2000; Shaikh et al. 2000, 2001; Stankiewicz and Lupski 2002*b*) (fig. 4). This LCR lies in the interval between the genetic markers D22S427 and D22S36/D22S1638 (Carlson et al. 1997; Edelmann et al. 1999*a,* 1999*b*). Telomeric to HIRA, however, the breakpoints of the microduplications appear to be different. Seven of the patients had a distal breakpoint between the genetic markers D22S1709 (CRKL [MIM 602007]) and D22S308, which bound the distal LCR implicated in the 3-Mb DG/VCFS deletion, suggesting that these patients have an ~3-Mb–sized duplication, which is similar in size to the deletion in 22q11.2 microdeletion syndromes.

The hybridization pattern in patient 8 and his mother and two sisters revealed a distal duplication breakpoint suggestive of a duplicated segment ~4 Mb in size, whereas patients 9 and 10 had patterns suggestive of a microduplication size of ~6 Mb (table 5; fig. 4). In addition to the proximal and distal LCRs implicated in the DG/VCFS 3-Mb deletion, other LCRs telomeric to the distal DG/VCFS LCR were recently identified on 22q (Edelmann et al. 1999*a,* 1999*b*). The presence of larger duplications, ~4 Mb in four of the patients and ~6 Mb in two of the patients, is an indication that these other LCRs, too, play an important role in rearrangements involving 22q.

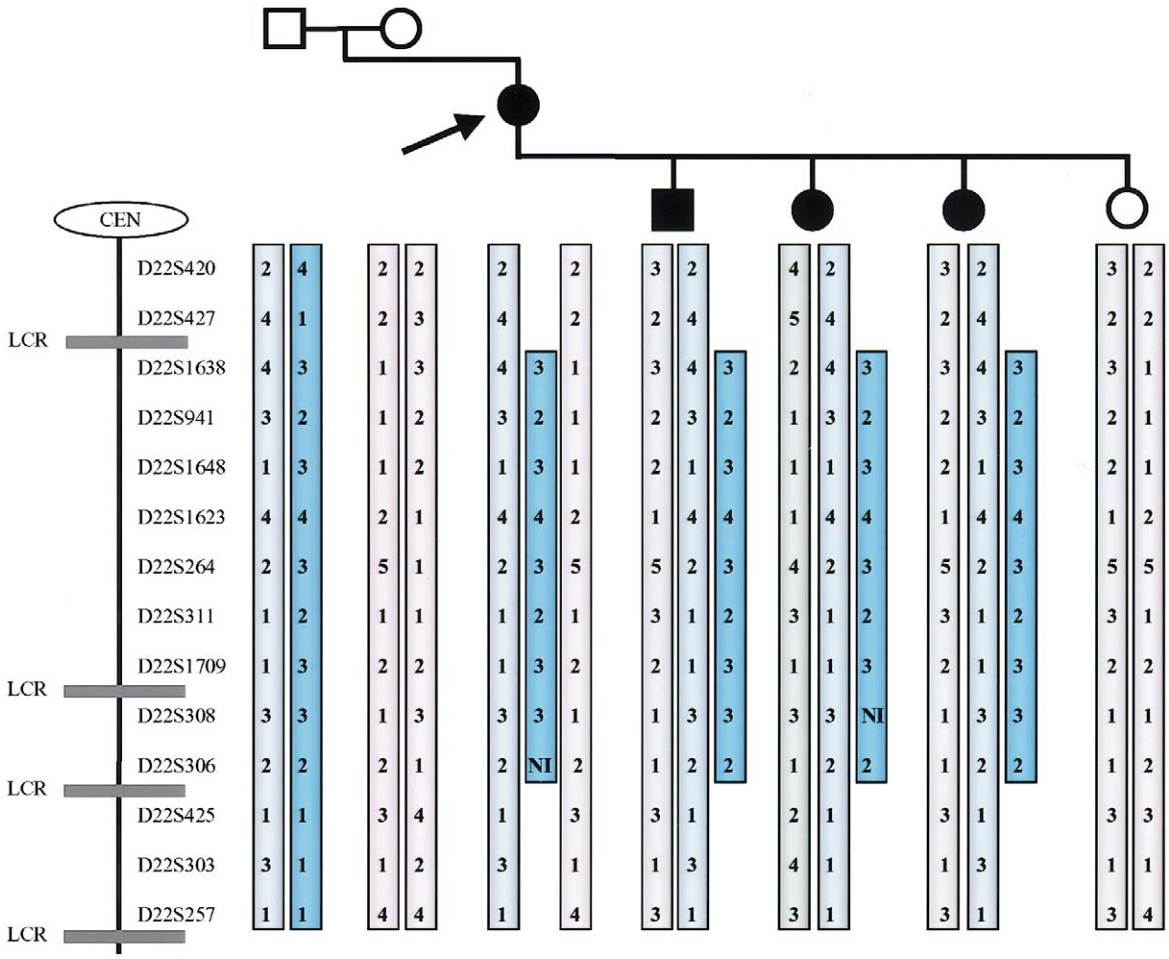

Figure 6 Results of genotyping of patient 8 and relevant family members through use of a panel of STR markers. Haplotype analysis indicates that the duplication occurred in the germline of the maternal grandfather.

Results of molecular analysis based on a panel of 15 STRs are in general agreement with the findings based on BACs and PACs. Through molecular analysis, we also discovered that each of the cases had three alleles for at least one STR (table 6; fig. 6). This would suggest that each of the microduplications observed was a segmental heterodisomy originating in the first meiotic division, although a precocious separation of sister chromatids in the first division of meiosis can also provide the same results. LCR-mediated microdeletion and microduplication are well documented for 17p, as well (Chance et al. 1994; Potocki et al. 2000). Recently, a family with three generations has been described in which the microduplication of 15q11.2-13 involved both maternal homologues in its origin from unequal crossing over in meiosis I, similar to our finding (Thomas et al. 2003).

The microdeletion sizes of 22q11.2 have ranged from 3 Mb (>90%) to 2 or 2.5 Mb, on the basis of 250 cases (Carlson et al. 1997; Kato et al. 2003), which implies that deletions >3 Mb are not tolerated. Since we are reporting microduplication sizes of 4 Mb and 6 Mb as well—although 3 Mb is most common—microduplications may prove to be more common than microdeletions. Our preliminary results do not support a correlation between the size of the duplication and the severity of the phenotypic presentation. This suggests that, in addition to gene dosage, other mechanisms—such as genetic and/or environmental interactions and, possibly, imprinting—may be important in determining the phenotypic outcome of patients with 22q11.2 microduplication.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for clones listed in table 5)
Genetic Information Research Institute, http://www.girinst.org/ (for the CENSOR server)
NCBI Home Page, http://www.ncbi.nlm.nih.gov/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for DG/VCFS, cat-eye syndrome, TUPLE1, ARSA, and CRKL)
UCSC Genome Bioinformatics Home Page, http://www.genome.ucsc.edu/ (for the genome browser)

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