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Der(22) Syndrome and Velo-Cardio-Facial Syndrome/DiGeorge Syndrome Share a 1.5-Mb Region of Overlap on Chromosome 22q11

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Summary

Derivative 22 (der[22]) syndrome is a rare disorder associated with multiple congenital anomalies, including profound mental retardation, preauricular skin tags or pits, and conotruncal heart defects. It can occur in offspring of carriers of the constitutional t(11;22)(q23;q11) translocation, owing to a 3:1 meiotic malsegregation event resulting in partial trisomy of chromosomes 11 and 22. The trisomic region on chromosome 22 overlaps the region hemizygously deleted in another congenital anomaly disorder, velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS). Most patients with VCFS/DGS have a similar 3-Mb deletion, whereas some have a nested distal deletion endpoint resulting in a 1.5-Mb deletion, and a few rare patients have unique deletions. To define the interval on 22q11 containing the t(11;22) breakpoint, haplotype analysis and FISH mapping were performed for five patients with der(22) syndrome. Analysis of all the patients was consistent with 3:1 meiotic malsegregation in the t(11;22) carrier parent. FISH-mapping studies showed that the t(11;22) breakpoint occurred in the same interval as the 1.5-Mb distal deletion breakpoint for VCFS. The deletion breakpoint of one VCFS patient with an unbalanced t(18;22) translocation also occurred in the same region. Hamster-human somatic hybrid cell lines from a patient with der(22) syndrome and a patient with VCFS showed that the breakpoints occurred in an interval containing low-copy repeats, distal to *RANBP1* and proximal to *ZNF74*. The presence of low-copy repetitive sequences may confer susceptibility to chromosome rearrangements. A 1.5-Mb region of overlap on 22q11 in both syndromes suggests the presence of dosage-dependent genes in this interval.

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Introduction

The constitutional t(11;22)(q23;q11) balanced translocation is the most common recurrent non-Robertsonian translocation in humans (Fraccaro et al. 1980; Zackai and Emanuel 1980). Although individuals who carry this translocation are normal, their offspring are susceptible to derivative 22 (der[22]) syndrome, owing to a 3:1 meiotic nondisjunction event. Affected offspring carry a supernumerary der(22) chromosome, 22pter-22q11:: 11q23-11qter (Fraccaro et al. 1980; Zackai and Emanuel 1980). The clinical features of patients with der(22) syndrome include severe mental retardation, malformed ears, preauricular skin tags and/or pits, cleft palate, micrognathia, and conotruncal heart defects (predominantly, atrial septal defect) (Zackai and Emanuel 1980; Lin et al. 1986). A second disorder of increased gene dosage on chromosome 22q11 is termed "cat-eye syndrome" (CES; MIM 115470). Patients with CES have a partial tetrasomy of chromosome 22 that is due to the presence of a bisatellited supernumerary chromosome with an inverted duplication of 22pter-22q11 (Schinzel et al. 1981; Mears et al. 1994, 1995). The duplication breakpoints of some patients with CES overlap with the region that is trisomic in patients with der(22) syndrome (McTaggart et al. 1998). The phenotypic spectrum of patients with der(22) syndrome overlaps with that of CES but is associated with more-severe mental retardation, presumably owing to the effects of trisomy 22q11.2 and 11q23-qter (Guanti 1981; Schinzel et al. 1981; Reiss et al. 1985; Van Hove et al. 1992; Knoll et al. 1995).

In addition to disorders that occur as a result of increased gene dosage, decreased gene dosage on 22q11 is responsible for the etiology of velo-cardio-facial syndrome (VCFS; MIM 192430) (Shprintzen et al. 1978; Driscoll et al. 1992*a;* Scambler et al. 1992) and DiGeorge syndrome (DGS; MIM 188400) (Driscoll et al. 1992*b;* Scambler et al. 1992). VCFS is characterized by velopharyngeal insufficiency, mild facial dysmorphology, submucous cleft palate, conotruncal heart defects, and learning disabilities (Shprintzen et al. 1978). DGS is phe-

notypically related to VCFS, and patients with DGS have, in addition to the clinical findings of VCFS, thymic aplasia or hypoplasia and severe hypocalcemia (DiGeorge 1965). Most patients with VCFS and DGS have overlapping hemizygous deletions of 22q11, suggesting that haploinsufficiency of the same genes may be responsible for the etiology of these diseases (Driscoll et al. 1992*a,* 1992*b;* Scambler et al. 1992). Haplotype analysis performed by use of 15 consecutive genetic markers on 22q11 showed that 83% of the patients with VCFS had a 22q11 deletion (Carlson et al. 1997); of these patients, 90% had a similar 3-Mb deletion. A second class of deletions share the 3-Mb proximal breakpoint but have a nested distal deletion endpoint resulting in a 1.5-Mb deletion. Although rare, a few patients with VCFS/DGS have unique rearrangements or deletions (Budarf et al. 1995; Kurahashi et al. 1996; Levy et al. 1995; O'Donnell et al. 1997). The 3-Mb region that is deleted in patients with VCFS/DGS overlaps with the region that is duplicated in some patients with CES (Mears et al. 1994, 1995; McTaggart et al. 1998) and in patients with der(22) syndrome (Carey et al. 1990; Budarf et al. 1996), suggesting that dosage-sensitive genes may reside in this interval.

To precisely define the interval containing the t(11;22) breakpoints on 22q11, we performed haplotype analysis and FISH-mapping studies of patients with der(22) syndrome. We found that the t(11;22) breakpoint occurs in the same interval as the 1.5-Mb distal deletion breakpoint for VCFS/DGS. These results suggest that there are sequences in this interval that are prone to rearrangement.

Subjects and Methods

Clinical Diagnosis and Preparation of DNA from Patients with Der(22) Syndrome and VCFS

The patients with der(22) syndrome who were ascertained for this study met the diagnostic criteria for the disorder, including most or all of the following clinical features: moderate to profound mental retardation, characteristic mild facial dysmorphology, preauricular skin tags or pits, congenital heart disease, genitourinary defects, renal defects, and the presence of the der(22) chromosome. BM85 is a male of Arabic descent and was examined most recently at 13 years of age. He was severely retarded, nonverbal, and aggressive and had widely spaced nipples, with an asymmetric chest, a normal heart, abnormal genitalia, and mild facial dysmorphology. Patient BM97, a black American, died shortly after birth. Her physical examination showed prominent bilateral preauricular fistula, typical facial dysmorphology, large anterior cataract on the right eye, clinodactyly and brachydactyly, malposition of the anus, with a tag, and widely spaced nipples. BM113, the dizygotic twin of BM97, was normal. BM317, a patient with der(22) syndrome, is of Greek descent and was examined at 17 mo of age. Examination showed a cleft palate, micrognathia, unilateral preauricular tags, and hip dysplasia. Cell lines were obtained for the two patients with der(22) syndrome, GM06228 and GM04370, from the NIGMS cell repository (Coriell Cell Repositories). The diagnostic criteria for VCFS have been described elsewhere (Carlson et al. 1997). Genomic DNA was prepared from 5 ml peripheral venous blood, by use of the Puregene protocol (Gentra). The blood samples were collected with the patients' informed consent (program approved by the Internal Review Board of the Albert Einstein College of Medicine).

Genotype Analysis

Each sample containing genomic DNA (100 ng) was genotyped separately with 15 consecutive genetic markers: *D22S420, D22S427, D22S1638, D22S941, D22S1648, D22S944N, D22S1623, D22S264, D22S311, D22S1709, D22S306, D22S308, D22S425, D22S303,* and *D22S257* (Carlson et al. 1997). One of two primers was radiolabeled, and a PCR product was amplified under standard conditions (Morrow et al. 1995). The radiolabeled PCR products were separated on 6% acrylamide denaturing sequencing gels, and a set of individuals with known allele sizes was used as a sizing control. The autoradiograms were exposed for 24 h, and films were analyzed manually. Mendelian inheritance within each family was verified.

FISH Mapping

The three LL22NC03 cosmids (Roswell Park Cancer Institute) that were used for FISH mapping were 62C4 (22q13), 84F7 (22q11), and 48C12 (22q11). The DNA (50 μg) from each cosmid was prepared by use of the Qiagen midi-preparation kit. The cosmid DNAs (1 μg/5 slides) were labeled separately by nick translation with either biotin-16 dUTP (Gibco BRL BioNick Labeling System), for 84F7 and 48C12, or digoxigenin-11-dUTP (Boehringer Mannheim Nick Translation Kit), for 62C4. The repetitive sequences on the cosmids were blocked by prehybridization with 50 μg Cot-1 DNA (Gibco BRL). Metaphase chromosomes were prepared as described elsewhere (Luan et al. 1997). Detection of nick-translated probes was performed as described in Oncor's "*In Situ* Hybridization Manual." In brief, for detection of biotinylated probes, successive incubations with fluorescein-labeled avidin and anti-avidin antibodies were performed. For detection of digoxigenin-labeled probes, successive incubations with rhodamine-labeled anti-digoxigenin, rabbit anti-sheep antibodies, and rhodamine-labeled anti-rabbit antibodies were performed. The

chromosomes were stained with propidium iodide (single-probe detection) or DAPI (dual-color detection).

Generation of Human-Hamster Somatic Hybrid Cell Lines

Somatic hybrid cell lines were developed by fusion of Epstein-Barr–transformed lymphoblastoid cell lines from patient BM97, as described elsewhere (Carlson et al. 1997). Somatic hybrid cell lines were generated previously for patient BM8, who had VCFS (Carlson et al. 1997). In brief, the lymphoblastoid cells from patient BM97 were fused, by use of polyethylene glycol, to hypoxanthine phosphoribosyltransferase–deficient CHTG49 Chinese hamster-ovary fibroblasts. After selection in HAT medium (hypoxanthine, aminopterin, and thymidine; Gibco/BRL), individual clones were tested, by PCR, for retention of chromosome 22. The positive clones were expanded and subjected to genotype analysis, to confirm the integrity of each copy of chromosome 22, as well as to identify the der(22) chromosome. Clones that contained either the normal chromosome 22 or the der(22) chromosome were expanded further, and DNA was extracted (Puregene Kit, Gentra).

Results

Haplotype Analysis

To define the region that contains the t(11;22) breakpoint, we performed haplotype analysis by using 15 ordered genetic markers that span a 5-Mb region on 22q11 (Carlson et al. 1997). The DNAs from five patients, BM85, BM97, BM317, GM06228, and GM04370, and their family members, when available, were genotyped. Haplotypes were deduced by comparison of the genotypes of the patients with der(22) syndrome with those of their parents (fig. 1). The presence of three alleles of different sizes for a given genetic marker is informative that the locus is trisomic. Inheritance of one allele from a heterozygous t(11;22) carrier parent and a different-sized allele from the other parent is informative for a disomic locus.

Three different alleles (6, 3, and 5) were detected for *D22S420* in der(22) syndrome patient GM06228 (fig.1). The patient had inherited alleles 3 and 5 from her t(11;22) carrier mother and allele 6 from her father (not available for analysis). For the next distal marker, *D22S427,* the patient had inherited alleles 1 and 4, as had her mother. This marker was uninformative. Markers *D22S427–D22S264* were uninformative for this patient. For the next distal marker, *D22S311,* the mother had alleles 4 and 1. The patient must inherit both alleles to be considered trisomic at this locus. Since the child was homozygous for allele 4, she must have inherited only one copy of *D22S311* from her mother. The t(11;22) breakpoint must have occurred proximal to this marker. Marker *D22S1709* was also disomic. The t(11;22) breakpoint occurred between *D22S420* and *D22S311.*

The haplotypes for three other patients with der(22) syndrome—GM04370, BM317, and BM97—are shown in figure 1. For patient GM04370, the t(11;22) breakpoint occurred between *D22S427* and *D22S264.* For BM317, the breakpoint was between *D22S941* and *D22S425,* and for BM97 the breakpoint was between *D22S1648* and *D22S264.* BM113, the dizygotic twin sister of BM97, was found to be a t(11;22) carrier, as deduced by means of haplotype analysis (fig. 1). Haplotype analysis could not be performed for der(22) syndrome patient BM85, because the DNA from his parents was not available for analysis. For BM85, three different-sized alleles were present for *D22S427* and *D22S1638,* and the rest of the markers had one or two different-sized alleles (data not shown).

To more precisely define the t(11;22) breakpoint in each patient and to determine whether it lies in the same interval, we performed the FISH-mapping studies described in the next section (figs. 2 and 3). The probes used for FISH were derived from cosmids 84F7 and 48C12. Cosmid 84F7 is distal to *D22S1623,* and cosmid 48C12 contains the *D22S264* marker (fig. 4). Because the breakpoints were found to occur in the interval flanked by the two cosmids, which is between *D22S1623* and *D22S264,* their locations were incorporated into figure 1. The interval flanked by *D22S1623* and *D22S264* is the same interval that contains the 1.5-Mb distal deletion breakpoint in VCFS patients BM8, BM13, BM14, and BM15, as was determined by haplotype analysis (Carlson et al. 1997). The results from haplotype analysis of VCFS patient BM58, who carries a t(18;22) unbalanced translocation, are shown in figure 1. The unbalanced-translocation breakpoint also occurred between *D22S1623* and *D22S264* (fig. 1).

FISH Mapping

FISH mapping was performed to more precisely map the t(11;22) breakpoint in the five patients described above. Representative FISH-mapping results, with two cosmid probes, 84F7 and 48C12, on the metaphase chromosomes from lymphoblastoid cell lines prepared from BM97 and her t(11;22) carrier mother, BM114, are shown in figure 2. The probes for FISH mapping were derived from two cosmids that map to the 22q11 region near *D22S1623* and *D22S264.* Cosmid 84F7 maps distal to *D22S1623,* and cosmid 48C12 contains *D22S264* (fig. 4). The two cosmid probes, when hybridized separately, produce one set of signals on each of the two normal copies of chromosome 22 and are deleted in patients with VCFS who have the common 3-

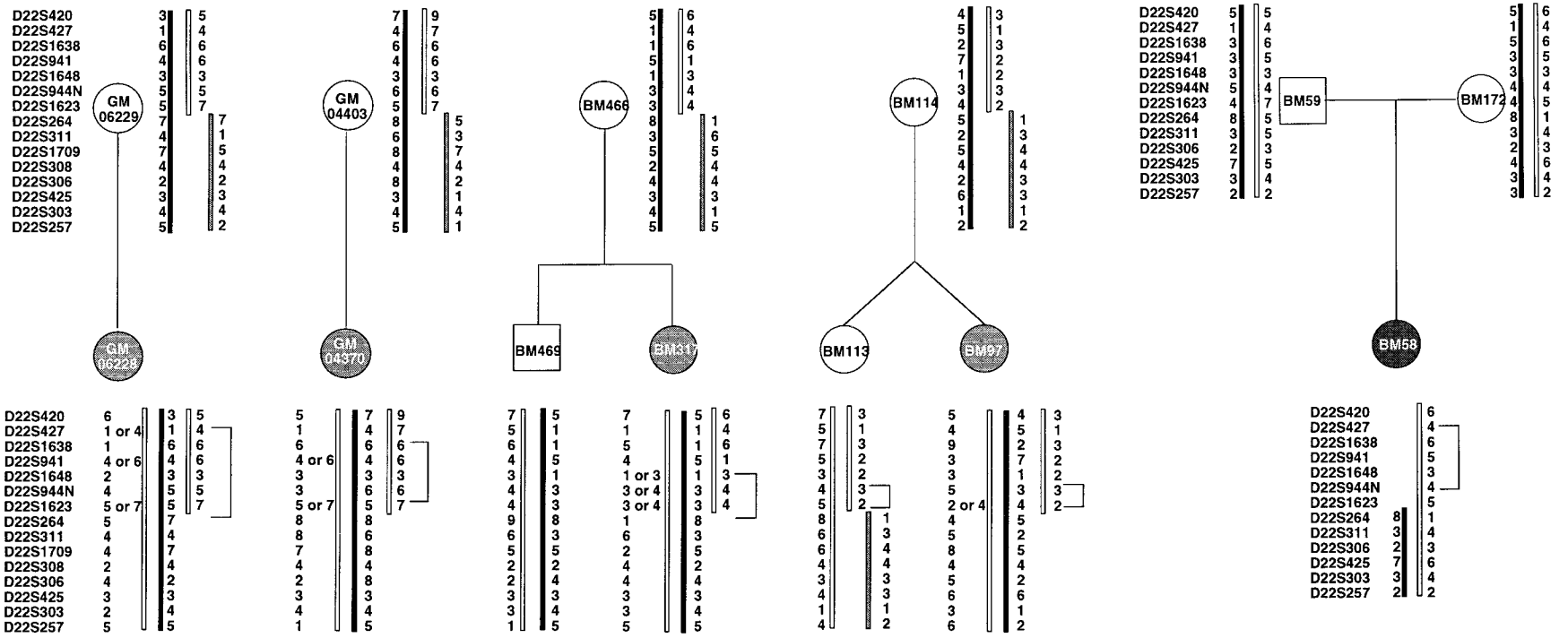

Figure 1 Haplotype analysis of patients with der(22) syndrome (*gray-shaded symbols*) and patients with VCFS (*blackened symbols*). VCFS patient BM58 had a t(18;22) unbalanced translocation. The 15 genetic markers that span the 22q11 region are ordered from the top, most centromeric marker, *D22S420*, to the bottom, most telomeric marker, *D22S257* (Carlson et al. 1997). The bars represent the chromosome of origin, as deduced from the genotype of the patient. A bracket indicates a region containing uninformative markers for the t(11;22) or VCFS deletion breakpoint interval. The chromosome breakpoints in each patient occurred between *D22S1623* and *D22S264*, as determined by FISH mapping (figs. 2 and 3).

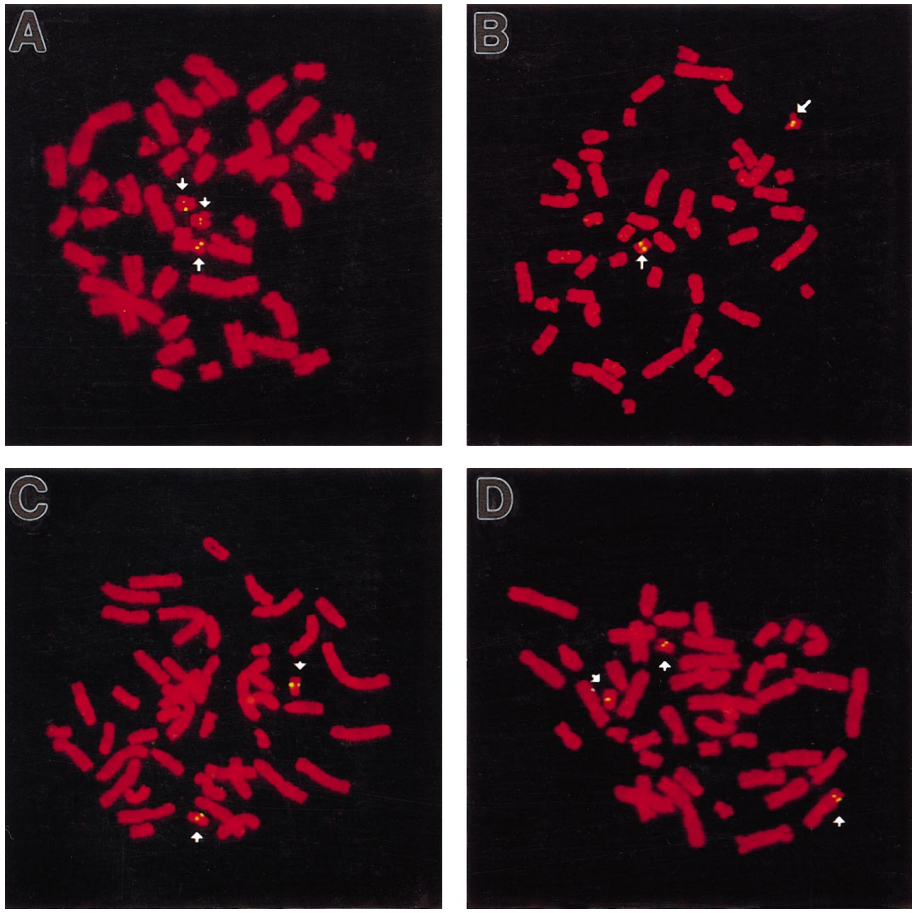

Figure 2 Results of FISH-mapping studies of BM97 and BM114. Metaphase chromosomes from BM97 (*A* and *B*) and her t(11;22) carrier mother, BM114 (*C* and *D*), were hybridized with fluorescent probes from cosmids 84F7 (*A, C,* and *D*) and 48C12 (*B* and *D*). Cosmid 84F7 maps proximal to cosmid 48C12 (see fig. 4).

Mb deletion (data not shown). For BM97, 84F7 hybridized to three different loci, as shown in figure 2*A*. Hybridization was detected on the der(22) chromosome and on each normal copy of chromosome 22. The more telomeric 48C12 probe hybridized to two loci, one on each normal chromosome 22 (fig. 2*B*). These results show that, for BM97, the cosmid probes flank the t(11;22) breakpoint. The same result was obtained for der(22) syndrome patients BM85, BM317, GM04370, and GM06228 (data not shown). For BM114, the t(11;22) carrier mother of BM97, two sets of signals were detected with 84F7 (fig. 2*C*). Hybridization occurred on the der(22) chromosome and on the normal copy of chromosome 22. When the two probes, 84F7 and 48C12, were mixed and hybridized to chromosomes from BM114, three signals were detected—one on the der(11) chromosome, one on the der(22) chromosome, and one on the normal copy of chromosome 22 (fig. 2*D*).

Similar FISH-mapping studies were performed on BM58, the patient with VCFS who carries a t(18;22) unbalanced translocation. A cosmid 62C4 probe that maps to 22q13 was used to identify the der(18) chromosome (fig. 3). Hybridization of 62C4 to normal metaphase chromosomes detects signals only on chromosome 22q13 (data not shown). In the metaphase spread from BM58, 62C4 hybridized (rhodamine detection) to the normal copy of chromosome 22 and to the larger der(18) chromosome (fig. 3*A*). Hybridization was detected, by use of 84F7 (fluorescein detection), on the normal copy of chromosome 22 but not on the der(18) chromosome (fig. 3*A*). Hybridization was detected, for 48C12 (fluorescein detection), on the larger der(18) chromosome and on the normal chromosome 22 (fig. 3*B*). These results indicate that the deletion occurred between cosmids 84F7 and 48C12.

Representative FISH-mapping results are shown for patient BM15 (fig. 3), a patient with VCFS and the 1.5-Mb deletion (Morrow et al. 1995). The 22q13 62C4 probe hybridized to both copies of chromosome 22, as

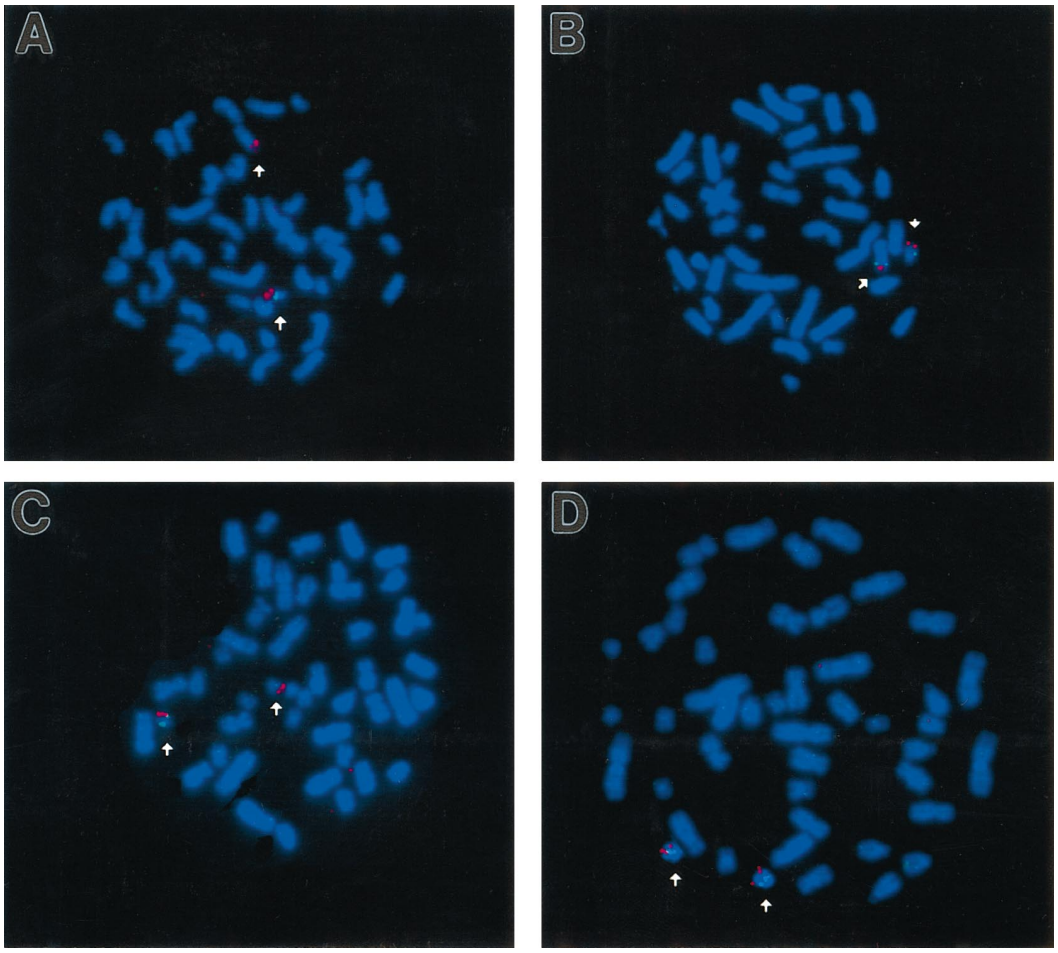

Figure 3 Results of FISH-mapping studies of two patients, BM58 and BM15, with VCFS. Metaphase chromosomes from the t(18;22) unbalanced translocations of VCFS patient BM58 (*A* and *B*) and unrelated VCFS patient BM15 (*C* and *D*), who has the 1.5-Mb deletion, were hybridized with probes from cosmids 84F7 and 62C4 (fluorescein and rhodamine, respectively; *A* and *C*) or cosmids 48C12 and 62C4 (fluorescein and rhodamine, respectively; *B* and *D*). Cosmid 62C4 maps to 22q13, near the telomere, and was used as a probe to detect chromosome 22.

was observed by the presence of two sets of signals (rhodamine detection; fig. 3*C*). Only one set of signals was detected for 84F7, on one of the two chromosomes 22 (fluorescein detection; fig. 3*C*). Two hybridization signals were detected, by use of 48C12, on both copies of chromosome 22 (fig. 3*D*), indicating that the distal deletion breakpoint for BM15 occurred between cosmids 84F7 and 48C12, in the same region as that in BM58. Similar results were obtained for patients AP1037 and GM05401 (data not shown). The FISH results showed that the t(11;22) translocation breakpoint and the VCFS 1.5-Mb distal deletion breakpoint occurred in an identical interval flanked by 84F7 and 48C12 (fig. 4). The genetic interval containing the breakpoints is flanked by *D22S1623* and *D22S264* (fig. 1).

Physical Map of the Breakpoint Interval

To define the 5-Mb 22q11 region, a physical map was constructed by assembling of overlapping sets of YACs, termed "YAC contigs" (Collins et al. 1995; Morrow et al. 1995). A gap in the physical map, owing to an underrepresentation of YAC clones, occurred between markers *D22S933* and *D22S264* (Collins et al. 1995; Morrow et al. 1995) (fig. 4). A high-resolution physical map of a 1,080-kb region in 22q11, consisting of overlapping cosmid clones, was constructed (Carlson et al. 1997). The distal end of the cosmid contig was demarcated by the sequence-tagged site (STS) *D22S933,* present in P1 artificial chromosome (PAC) 296F16 (Carlson et al. 1997). To extend the physical map in a telomeric direction, we developed STS markers from the genomic sequence of cosmid 68F10 and PAC 201M18 (University of Oklahoma; fig. 4). The STSs were used as probes to obtain additional clones for genomic walking. Genomic walking was used to extend the map in a centromeric direction from the genetic marker *D22S264*. Positive bacterial artificial chromosome (BAC), PAC, and cosmid

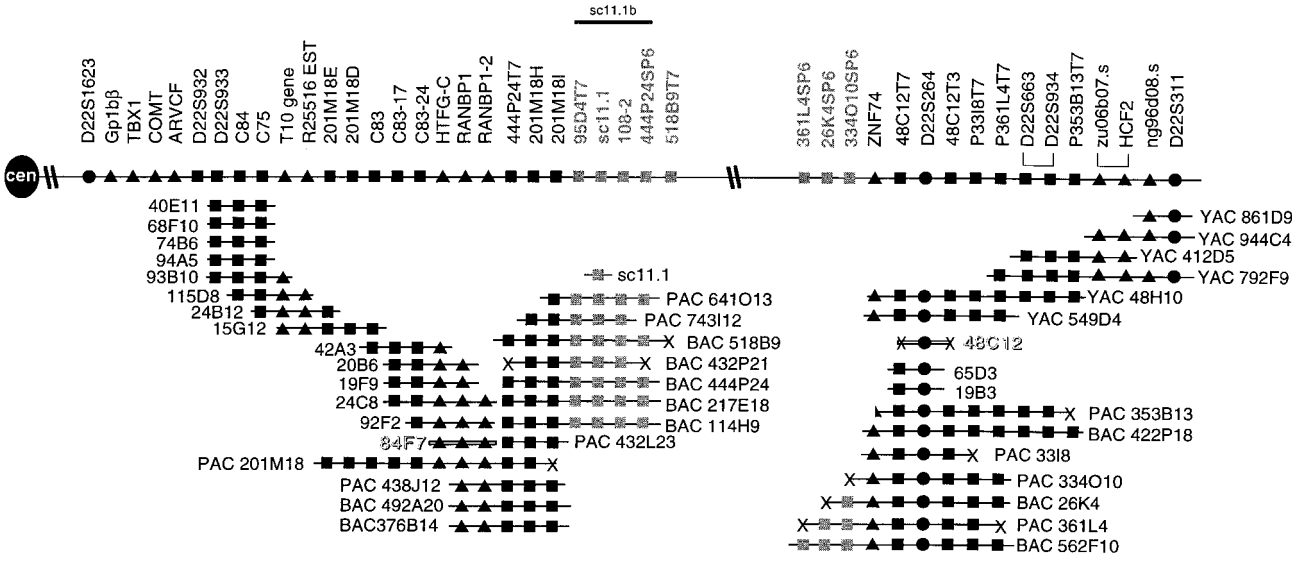

Figure 4 High-resolution physical map of the chromosome breakpoint interval on 22q11. The PCR markers used to construct the map are indicated above the line representing chromosome 22q11. The orientation of the map is centromere (*left*) to telomere (*right*). The genetic markers (circles), anonymous genomic markers (squares), and genes or expressed sequences (triangles) are shown. STS markers were developed from the genomic sequences of cosmid 68F10 and PAC 201M18 (University of Oklahoma). We developed a set of STS markers that spanned this region from the sequence. The STSs were used as probes, to obtain additional clones for genomic walking. The STSs derived from the ends of clones are denoted by "X." The gray-shaded symbols indicate low-copy repeats that are repeated elsewhere on 22q11. The bracket under markers *D22S663* and *D22S934* indicates that these markers could not be ordered unambiguously, with respect to each other. The bacterial clones represented below the line consist of BACs ("BAC" preceding the address), PACs ("PAC" preceding the address), and cosmids (nothing preceding the address). The cosmid clones used for FISH mapping are outlined.

clones were confirmed by PCR analysis. To increase the density of markers on the physical map, we sequenced the insert ends of bacterial clones and generated new PCR markers (fig. 4). The 84F7 and 48C12 cosmid clones used for FISH mapping were integrated into the map. Cosmid 84F7 is distal to genetic marker *D22S1623,* and cosmid 48C12 contains genetic marker *D22S264,* as shown in figure 4. The *T10* (Halford et al. 1993*b*), *RANBP1* (Bischoff et al. 1995; Puech et al. 1997), *HTF9-C* (Puech et al. 1997; Di Matteo et al. 1998), *ZNF74* (Aubry et al. 1993), and *HCF2* (Herzog et al. 1991) genes were integrated into the map (fig. 4). Of interest, one of the expressed-sequence tags, zu06b07.s, was homologous to the phosphatidylinositol 4–kinase gene, indicating that this gene may be localized to this interval (GenBank accession number AF012872).

Low-copy–repeat clusters span the 22q11 region (Halford et al. 1993*a;* Collins et al. 1997; L. Edelmann, unpublished data). One such low-copy repeat can be detected by interphase FISH, by use of a probe derived from a cosmid termed "sc11.1" (Lindsay et al. 1993). The sc11.1 cosmid probe produces two signals, sc11.1a (proximal) and sc11.1b (distal), that are 1–2 Mb apart on each normal copy of chromosome 22 (Lindsay et al. 1993). Both signals are deleted in patients with the 1.5-Mb or the 3-Mb VCFS deletion (Lindsay et al. 1995). The sc11.1a locus maps just distal to the common proximal deletion breakpoint that occurs in patients with VCFS (Carlson et al. 1997). The sc11.1b locus has been integrated into the physical map illustrated in figure 4. The sc11.1b locus maps telomeric to 201M18I and contains markers 95D4T7–444P24SP6 (fig. 4).

Markers 518B9T7, 361L4SP6, 26K4SP6, and 334O10SP6 are members of the 22q11 low-copy–repeat clusters termed the "LCR22s," which map to the proximal and distal deletion breakpoints in patients with VCFS/DGS who have the common 3-Mb deletion (Edelmann et al., in press). A set of genes or pseudogenes, including *GGT* (gamma-glutamyl transpeptidase gene) and *BCRL* (breakpoint cluster region–like gene) (Collins et al. 1997) compose each of the eight known LCR22s (Collins et al. 1997; L. Edelmann, unpublished data). To extend the physical map from the flanking repeat markers, it was necessary to identify clones that are anchored to this interval and that are positive for both markers 201M18I and *ZNF74.* None of the clones that were identified spanned this interval. To complete the physical map, it was necessary to identify BAC and PAC clones that are anchored to the physical map and that contain additional markers, from within the repeat, that can serve as additional anchor points for the assigning of clones to this specific repeat and not to the other eight

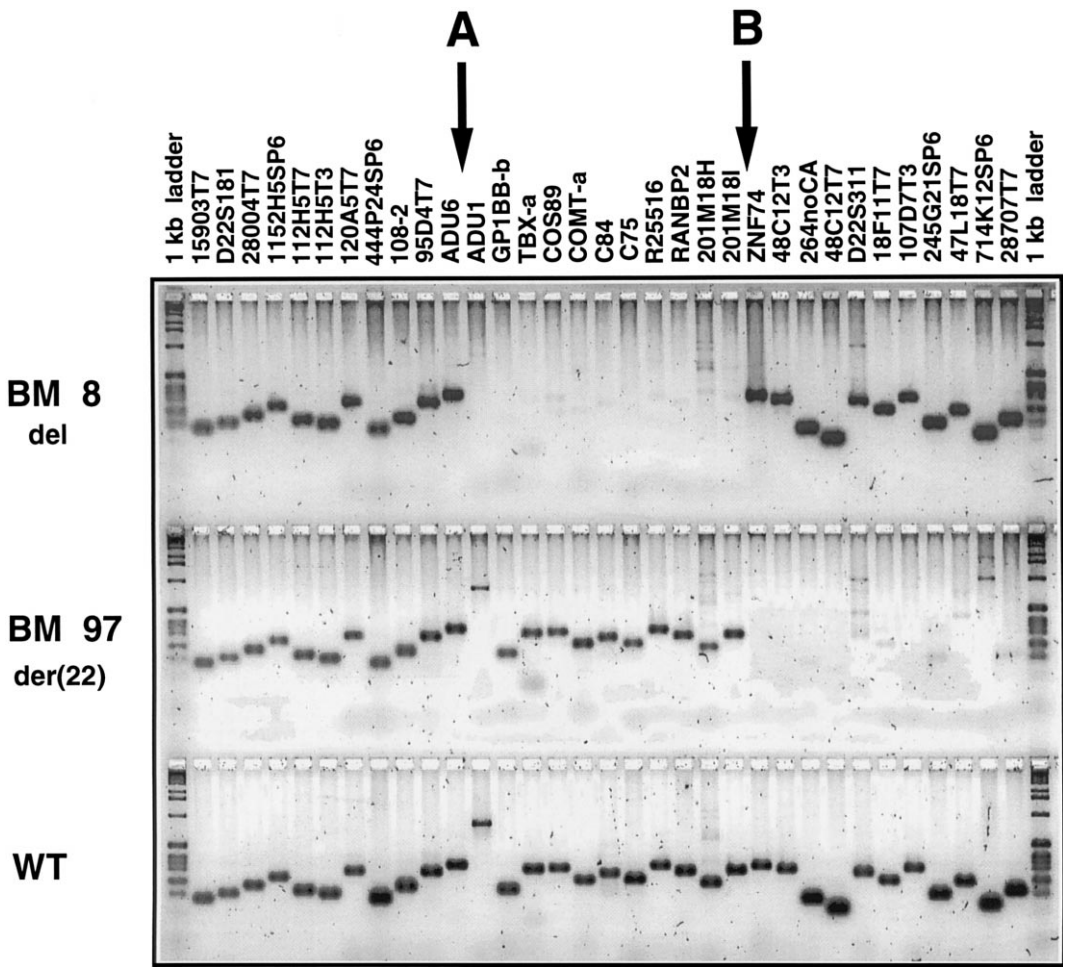

Figure 5 PCR analysis of chromosome 22q11 from hamster-human somatic hybrid cell lines. The deleted copy of chromosome 22q11 is shown for BM8, a VCFS patient. The der(22) chromosome and one intact chromosome from BM97 is also shown. The PCR markers that represent the 3-Mb chromosome 22q11 region are shown, ordered from the centromere (*left*) to the telomere (*right*). PCR markers 159O3T7–120A5T7 map proximal to the VCFS 3-Mb common proximal breakpoint (Edelmann et al., in press). Markers 444P24SP6, 108-2, and 95D4T7 compose the sc11.1a and sc11.1b loci (Edelmann et al., in press). ADU6 and ADU1 are 10,250 bp apart (Genbank AC000095) and contain the proximal breakpoint in BM8 and the ADU breakpoint. Markers GP1BB-b, TBX-a, COS89, COMT-a, 201M15H, and 201M15I were generated from the genomic sequence of 22q11 (University of Oklahoma). Markers C84–*D22S311* are shown in figure 4. Markers 18F11T7 and 107D7T3 (D22S1709) and markers 245G21SP6–287O7T7 map proximal and distal, respectively, to the VCFS 3-Mb distal deletion breakpoint interval (Edelmann et al., in press). The 1-kb ladder size standard (NE Biolabs) flanks the PCR products. Arrow A indicates the proximal breakpoint in BM8, and arrow B indicates the t(11;22) breakpoint and the distal deletion breakpoint interval in BM8. The 2% agarose gel was stained with SYBR green (Molecular Probes).

copies of LCR22s. The BAC and PAC libraries were underrepresented in this region. None of the cosmid clones that were isolated could be anchored to this specific copy of the LCR22s. Because of the repetitive nature of the markers in this interval, it has not yet been possible to close the remaining gap in the map that contains the repeat.

Somatic Cell–Hybrid Cell Analysis of the t(11;22) Breakpoint on 22q11

The average spacing of the 15 genetic markers that map to the 5-Mb 22q11 region is 300 kb. To more precisely define the interval containing the t(11;22) breakpoint on 22q11, we generated hamster-human somatic cell hybrids from BM97. The der(22) chromosome was physically separated from the two normal copies of chromosome 22 in separate cell lines. Template DNA from the der(22) chromosome was examined by PCR with the STS markers that were ordered on the physical map (fig. 5). The t(11;22) breakpoint occurred between the genes *RANBP1* and *ZNF74*, distal to 201M18I. The PCR markers distal to 201M18I and proximal to *ZNF74* were present in the DNA from the der(22) chromosome. However, because they were repeated on

22q11 in each copy of the LCR22s (L. Edelmann, unpublished data), they were not informative (fig. 5).

To determine whether patients with VCFS have a deletion breakpoint in the same region as the t(11;22) breakpoint on 22q11, a similar analysis was performed with the deleted copy of chromosome 22 from VCFS patient BM8 and with a normal copy of chromosome 22 from BM97, as a control (fig. 5). The common proximal breakpoint in patients with VCFS with the 1.5-Mb or the 3-Mb deletion occurs between *D22S427* and *D22S1638* (Carlson et al. 1997). Patient BM8 has a unique nested proximal deletion breakpoint within 40 kb distal to genetic marker *D22S1638* (Carlson et al.1997). The breakpoint in BM8 is between markers ADU6 and ADU1 (*A* in fig. 5). The distal deletion endpoint occurred between *D22S1623* and *D22S264* (Carlson et al. 1997). PCR analysis showed that the distal deletion breakpoint in BM8 occurred in the same interval as that for the t(11;22) breakpoint, between 201M18I and *ZNF74* (*B* in fig. 5). Similarly, the repetitive markers were not informative by PCR; however, interphase FISH studies with cosmid sc11.1 showed that one locus, presumably the sc11.1b locus, was deleted (Lindsay et al. 1995), indicating that the breakpoint in BM8 occurs distal to the sc11.1b locus and proximal to *ZNF74*.

Discussion

Mechanism of Rearrangements on 22q11

The 22q11 region is prone to rearrangements leading to congenital anomaly disorders. CES, der(22) syndrome, and VCFS/DGS occur as a result of tetrasomy, trisomy, and monosomy, respectively, of part of 22q11. The human genome contains repetitive DNA sequences that are categorized depending on their frequency of occurrence and sequence composition. For example, *Alu* elements are repeated several hundred thousand times, and long interspersed repetitive elements are repeated one order of magnitude less, throughout the genome (Moyzis et al. 1989). Sequences that are repeated just a few times in the genome, such as gene-family members, are referred to as low-copy repeats. Low-copy–repeat clusters of different sequence composition span the 22q11 region (Halford et al. 1993*a;* Lindsay et al. 1993; Collins et al. 1997).

Most patients with VCFS/DGS have a similar 3-Mb deletion. To understand the basis of the deletion, the region surrounding the deletion breakpoints was defined by construction of high-resolution physical maps. We found that the proximal and distal breakpoints leading to the common 3-Mb deletion occur in a low-copy–repeat cluster, the LCR22s (L. Edelmann, unpublished data). Both intrachromosomal rearrangements and unequal crossing over of misaligned chromosomes mediate the common 3-Mb deletion (Baumer et al. 1998). It is possible that the LCR22s mediate the meiotic homologous recombination events.

Approximately 7% of patients with VCFS have a smaller 1.5-Mb deletion (Morrow et al. 1995; Carlson et al. 1997). They share a common proximal breakpoint with the patients with VCFS who have the 3-Mb deletion but have a nested distal deletion breakpoint. In this study, we showed by haplotype analysis, FISH, and somatic cell–hybrid analysis that the t(11;22) translocation breakpoint occurs in the same interval as the 1.5-Mb distal VCFS deletion breakpoint. We also showed that LCR22 sequences map to the 1.5-Mb distal deletion breakpoint interval. It has not yet been possible to complete the map containing LCR22 sequences near the 1.5-Mb distal deletion breakpoint, because the anchored clones are not large enough to span the LCR22s. Because LCR22 sequences are present in the interval, it is possible that mechanisms of deletion that are similar to those for the 3-Mb deletion may occur. Further physical-mapping experiments and haplotype analysis of three-generation families of patients with the 1.5-Mb deletion are necessary to show whether the mechanism of the deletion is the same as that for patients with the 3-Mb deletion.

Low-copy repeats have been implicated in rearrangements associated with disorders that map to other chromosomes, such as Charcot-Marie-Tooth disease type 1A and hereditary neuropathy with liability to pressure palsies. These disorders are peripheral neuropathies associated with a duplication or deletion, respectively, of the same region on chromosome 17p11.2-12 (Chance et al. 1994). Other chromosomal rearrangement disorders associated with low-copy repeats of varying sizes are Smith Magenis syndrome on 17p11.2 (Chen et al. 1997), Prader-Willi syndrome/Angelman syndrome on 15q11-13 (Wandstrat et al. 1998), and Williams-Beuren syndrome on 7q11.23 (Perez Jurado et al. 1998). In most cases, the mechanism of rearrangement is by homologous recombination between low-copy repeats, as proposed for the 1.5-Mb and 3-Mb VCFS deletions.

The mechanism leading to the recurrent t(11;22) translocation is not known. The 11q23 interval that harbors the t(11;22) translocation has not been defined at the same resolution as for the 22q11 region. One possible reason why this translocation occurs recurrently in the population is that there may be a subset of low-copy–repeat sequences on 11q23 that are similar in composition to those on 22q11, and these sequences may mediate the balanced translocation, by homologous recombination events.

Dosage-Sensitive Genes Map to 22q11

CES, der(22) syndrome, and VCFS/DGS are clinical disorders associated with partial tetrasomy, trisomy, and

monosomy, respectively, of part of chromosome 22. The three disorders share a 1.5-Mb region of sequence overlap that is flanked by genetic markers *D22S427* and *D22S264*. Decreased gene dosage on 22q11 is associated with craniofacial anomalies, conotruncal heart defects, and learning disabilities. To determine whether increased gene dosage in this region contributes to the etiology of congenital anomalies, it is necessary to determine whether there are specific defects in individual patients that can be attributed to this interval.

The main clinical findings for patients with CES include mild or no mental retardation, deformed ears, preauricular skin tags and/or pits, and conotruncal heart defects (Guanti 1981). Two distinct CES-chromosome duplication breakpoints in 22q11 have been defined (McTaggart et al. 1998). The smaller, CES-I duplication breakpoint occurs in the same region as the common proximal breakpoint in patients with VCFS/DGS. Therefore, patients with CES-I do not share sequence overlap with patients with VCFS/DGS. The larger, CES-II duplication breakpoint occurs in the same region as the 3-Mb distal deletion breakpoint (McTaggart et al. 1998). Patients with CES-II share a 3-Mb region of overlap with patients with VCFS/DGS. If increased gene dosage in the 3-Mb region is associated with physical anomalies, patients with CES-II should have these additional anomalies. Clinical analysis of a small set of patients with CES-II showed that they are no more severely affected than are patients with CES-I, suggesting that the genes that contribute to CES are proximal to the CES-I breakpoint (McTaggart et al. 1998). The levels of phenotypic expression in patients with CES vary significantly, precluding the ability to precisely define chromosome subregions that contribute to the physical anomalies of this disorder (Mears et al. 1994; McTaggart et al. 1998).

The clinical features of CES overlap with those of patients with der(22) syndrome (Reiss et al. 1985; Knoll et al. 1995). In contrast to patients with CES, who have no or mild mental retardation, patients with der(22) syndrome have profound mental retardation (Reiss et al. 1985). To determine the basis of der(22) syndrome, it is necessary to identify the phenotypic contribution of the 22q11 and the 11q23-qter regions. Clinical assessment of anomalies in patients with rare 22q11 duplications suggests that trisomy 22q11 may contribute to the anomalies of der(22) syndrome (Reiss et al. 1985; Knoll et al. 1995; Lindsay et al. 1995; Prasher et al. 1995). However, the size of the duplications extend into the 22q12 or 22q13 regions (Reiss et al. 1985; Knoll et al. 1995; Lindsay et al. 1995; Prasher et al. 1995). It is possible that the 11q23-qter region may contribute to the severity of mental retardation in patients with der(22) syndrome (Van Hove et al. 1992; Smeets et al. 1997).

We recently have identified a child with developmental delay that has a duplication of the same 3-Mb region on 22q11 that is deleted in patients with VCFS/DGS and that is duplicated in patients with CES-II (L. Edelmann, unpublished data). These results suggest that increased gene dosage could contribute to the etiology of the developmental delay in this patient. The patient's mother and grandmother have the same duplication but are phenotypically normal, suggesting variable penetrance and a mild phenotype. Alternatively, the developmental delay in this patient could have a different basis, independent of the duplication of 22q11. To distinguish between these two possibilities, it will be necessary to ascertain additional patients with duplications of the 22q11 region who do not have other chromosomal abnormalities.

More than 20 genes and expressed sequences have been isolated from the 1.5-Mb 22q11.2 region that is shared by CES-II, der(22) syndrome, and VCFS/DGS. One way to assess the role of increased or decreased gene dosage in the etiology of congenital anomalies is to generate mice with a similar deletion or duplication, respectively, of the region of synteny in the mouse (Ramirez-Solis et al. 1995; Smith et al. 1995; Van Deursen et al. 1995). The 1.5-Mb region on the 22q11 region is conserved, and it maps to mouse chromosome 16 (Puech et al. 1997). On the basis of the high level of conservation between mice and humans, it may be possible to generate nested deletions and duplications that span the entire interval of overlap between the two disorders. By means of this approach, the molecular basis of VCFS, as well as the contributions of the deleted region to der(22) syndrome and CES, can be determined precisely.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Coriell Cell Repositories, NIGMS cell repository, http://arginine.umdnj.edu/ccr.html (for cell lines from two patients [GM06228 and GM04370] with der[22] syndrome)

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for the

phosphatidylinositol 4–kinase gene [AF012872] and for ADU6 and ADU1 [AC000095])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for CES [MIM 115470], VCFS [MIM 192430], and DGS [MIM 188400])

University of Oklahoma, map of DiGeorge region of human chromosome 22 and syntenic mouse chromosome 16, http://www.genome.ou.edu/maps/dgcr.html

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