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Molecular Analysis of Velo-Cardio-Facial Syndrome Patients with Psychiatric Disorders

C. Carlson,[1] D. Papolos,[2,3] R. K. Pandita,[1] G. L. Faedda,[2] S. Veit,[2] R. Goldberg,[1,4] R. Shprintzen,[4] R. Kucherlapati,[1] and B. Morrow[1]

[1]Department of Molecular Genetics, Albert Einstein College of Medicine of Yeshiva University; [2]Department of Psychiatry, Program in Behavioral Genetics, Albert Einstein College of Medicine of Yeshiva University/Montefiore Medical Center; [3]Department of Psychiatry, Division of Child Psychiatry, Albert Einstein College of Medicine of Yeshiva University/Bronx Children's Hospital Center; and [4]Center for Craniofacial Disorders of Montefiore Medical Center and Albert Einstein College of Medicine, Bronx

Summary

Velo-cardio-facial syndrome (VCFS) is characterized by conotruncal cardiac defects, cleft palate, learning disabilities, and characteristic facial appearance and is associated with hemizygous deletions within 22q11. A newly recognized clinical feature is the presence of psychiatric illness in children and adults with VCFS. To ascertain the relationship between psychiatric illness, VCFS, and chromosome 22 deletions, we evaluated 26 VCFS patients by clinical and molecular biological methods. The VCFS children and adolescents were found to share a set of psychiatric disorders, including bipolar spectrum disorders and attention-deficit disorder with hyperactivity. The adult patients, >18 years of age, were affected with bipolar spectrum disorders. Four of six adult patients had psychotic symptoms manifested as paranoid and grandiose delusions. Loss-of-heterozygosity analysis of all 26 patients revealed that all but 3 had a large 3-Mb common deletion. One patient had a nested distal deletion and two did not have a detectable deletion. Somatic cell hybrids were developed from the two patients who did not have a detectable deletion within 22q11 and were analyzed with a large number of sequence tagged sites. A deletion was not detected among the two patients at a resolution of 21 kb. There was no correlation between the phenotype and the presence of the deletion within 22q11. The remarkably high prevalence of bipolar spectrum disorders, in association with the congenital anomalies of VCFS and its occurrence among nondeleted VCFS patients, suggest a common genetic etiology.

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Address for correspondence and reprints: Dr. Bernice Morrow, Department of Molecular Genetics, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail: morrow@aecom.yu.edu

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Introduction

Velo-cardio-facial syndrome (VCFS) is an autosomal dominant multiple anomaly disorder with an estimated frequency of 1/4,000 live births (Burn and Goodship 1996). The main physical anomalies of VCFS include conotruncal cardiac malformations, cleft palate, learning disabilities, and a characteristic facial appearance (Shprintzen et al. 1978). Since its original description in 1978, >40 physical anomalies with variable levels of phenotypic expression have been observed in association with VCFS (Goldberg et al. 1993). These include hypotonia, lymphoid tissue hypoplasia, transient neonatal hypocalcemia, slender hands and digits, small stature, mild mental retardation, microcephaly, and tortuous retinal vessels (Young et al. 1980; Kelley et al. 1982; Jedele et al. 1992; Goldberg et al. 1993). The phenotypic spectrum of VCFS overlaps with that of DiGeorge syndrome (DGS) (Goldberg et al. 1985; Stevens et al. 1990), originally described by DiGeorge (1965). Many of the tissues and structures affected in VCFS and DGS are derived from the pharyngeal arches of the developing vertebrate embryo (Le Douarin 1980; Kirby et al. 1983; Kirby and Bockman 1984; Bockman and Kirby 1989; Kirby and Waldo 1995), suggesting that the gene(s) responsible for VCFS and DGS are required for normal embryonic development.

A large proportion of VCFS and DGS patients are hemizygous for part of 22q11 (de la Chapelle et al. 1981; Kelley et al. 1982; Augusseau et al. 1986; Rouleau et al. 1989; Scambler et al. 1992), suggesting that haploinsufficiency of a gene(s) within 22q11 is responsible for VCFS and DGS. It is possible that VCFS and DGS have the same molecular etiology. To define the region within 22q11 that is deleted among patients, we constructed a physical map in the form of overlapping YACs. This map contains, among other PCR-based markers, a set of 14 ordered simple tandem repeat polymorphic (STRP) markers. The STRP markers were used to define the extent of the deletion in each patient by detecting a loss of heterozygosity of a particular marker when compared to the unaffected parents. In a group

of 15 VCFS patients and their unaffected parents who were previously examined by haplotype analysis using 11 STRP markers (Morrow et al. 1995), we detected two major classes of deletions. The first, which occurred most frequently, is 3 Mb in length, and the second, which occurred in fewer patients, is ~1.5 Mb in size. The smaller deletion, which is nested within the larger one, is flanked by STRP markers D22S427 and D22S264. It is likely that haploinsufficiency of one or more genes within this interval is responsible for the physical anomalies associated with VCFS.

A newly appreciated clinical feature of VCFS is that a significant number of the adult patients develop psychiatric illness (Goldberg et al. 1993). Some of the early diagnoses given to these patients included mood or affective disorders such as bipolar disorder, obsessive compulsive disorder, depression, and schizophrenia (Goldberg et al. 1993; Chow et al. 1994; Pulver et al. 1994; Karayiorgou et al. 1995). Developmental studies of children with VCFS revealed early signs of psychiatric problems (Golding-Kushner et al. 1985). Because of the possibility that children with VCFS are prodromal for psychological disorders, we recruited 26 VCFS patients (ages 5–34 years) for psychiatric evaluation. We observed that a common spectrum of bipolar spectrum disorders occur in children with VCFS (Papolos et al. 1996). Attention-deficit disorder with hyperactivity (ADHD) and bipolar spectrum disorders were the most common diagnoses among children and adult patients, respectively.

To define the extent of deletions that occur in the 26 VCFS patients and to correlate the deletion with the phenotype, we performed haplotype analysis using a series of 14 consecutive STRP markers. Most of the VCFS patients had the commonly occurring 3-Mb deletion. One patient had a nested distal deletion breakpoint, and two patients did not have a detectable deletion. The smallest region of deletion overlap among the patients was an interval of 1.5 Mb flanked by the genetic markers D22S427 and D22S264. Within this group of patients, there was no correlation between the severity of the disorder and the size or presence of a deletion within 22q11. These results suggest that the genetic factors leading to the physical anomalies of VCFS may also be responsible for the psychiatric phenotype.

Methods

Selection and Clinical Evaluation of the VCFS Patients

The subjects for the psychiatric evaluation were chosen randomly as they returned for annual VCFS follow-up examinations, with the exception of AP1012, AP1018, AP1035, and AP1079, who were previously evaluated for psychiatric disorders and agreed to participate in the study (patients 1, 6, 11, and 14; Pulver et al. 1994). The criteria for selecting the subjects included diagnosis with VCFS at the Center for Craniofacial Disorders at the Montefiore Medical Center, age ≥ 5 years, and written informed consent from patients and/or parents. The diagnostic criteria for VCFS included, at minimum, the characteristic VCFS facies, hypernasal speech, submucous cleft palate, velo-pharyngeal insufficiency, and evidence of mild learning disabilities during childhood (Shprintzen et al. 1978, 1981; Goldberg et al. 1993). The physical characteristics of the VCFS patients, including the facies and hypernasal speech, prevented the interviewers from being blind to the VCFS diagnosis. However, they were blind to the 22q11 deletion status. Each of the children and adolescents with VCFS were evaluated for psychiatric disorders by use of the Diagnostic Interview for Children and Adolescents—Parent and Child Versions—Revised (DICA-R-P, DICA-R-C) (Herjanic and Campbell 1977; Herjanic and Reich 1982; Reich and Welner 1985). The Standardized Clinical Diagnostic Interview (SCID) was used for patients ≥ 18 years of age (Williams et al. 1992). Both the DICA and SCID interviews were computerized and self-administered. By definition, the computerized interview is structured. A clinical interview followed the review of preliminary diagnoses elicited by the structured interviews (DICA-R-P, DICA-R-C, and SCID), as well as all previous psychiatric records, and was administered to validate and elucidate the chronological appearance, duration, and intensity of specific symptoms and syndromes reported by the DICA-R-P, DICA-R-C, and SCID interviews (Papolos et al. 1996).

Human Tissue Samples, Cell Lines, and DNA Extraction

Blood samples from 26 VCFS patients and their parents were collected, in an institutional review board–approved program, and DNA was prepared as described by Morrow et al. (1995). Epstein Barr virus (EBV)–transformed lymphoblastoid cell lines were established (Morrow et al. 1995) from the lymphoblasts of each of the patients.

Polymorphic Marker Analysis

Polymorphic marker analysis was performed as described (Morrow et al. 1995). The ordered set of markers that were used for this analysis are D22S420, D22S427, D22S1638, D22S941, D22S1648, D22S944, D22S1623, D22S264, D22S311, D22S306, D22S308, D22S425, D22S303, and D22S257 (Morrow et al. 1995; B. Morrow and C. Carlson, unpublished data). The DNA template (100 ng) prepared from lymphoblast cell lines or peripheral blood was used in a 10-μl PCR reaction. The conditions for PCR are identical in each case and are described (Morrow et al. 1995).

Somatic Cell Hybrids

Somatic cell hybrids were developed by fusion of EBV-transformed lymphoblastoid cell lines from patients BM26 and BM102 with the hypoxanthine phosphoribosyl transferase–deficient Chinese hamster ovary (CHO) fibroblast cell line GM 10658 according to well-established methods. Hybrid cell lines, which grew in the presence of medium containing hypoxanthine, aminopterin, and thymidine, were isolated. Large-scale cultures consisting of 1.5×10^8 cells from each cell line were used for preparing DNA (Puregene DNA kit; Gentra) with an average yield of 1.5 mg/preparation. This DNA was used for genotyping and for PCR-based sequence-tagged-site (STS) content analysis.

Results

Physical and Psychiatric Assessment of the VCFS Patients

A summary of each of the physical and psychiatric findings of each patient is presented in table 1. Details of the psychiatric diagnoses have been described by Papolos et al. (1996). All but one of the patients received a psychiatric diagnosis. We observed variation in the phenotypic spectrum of both the physical and psychiatric findings in association with VCFS. Half of the patients in this group had conotruncal heart or great vessel defects. There were six children in this study who were $\leq$10 years of age. Four of these children were diagnosed to have attention-deficit disorder (ADD) or ADHD. Nineteen patients >11 years of age were affected with bipolar spectrum disorders. Four of the six patients >20 years of age had psychotic symptoms that included paranoid and grandiose delusions.

FISH Analysis for 22q11 Deletions

To ascertain the status of 22q11 in the 26 patients, cell lines derived from peripheral blood lymphocytes were used for high-resolution karyotypic analysis and for FISH. Analysis by FISH was conducted with either or both of the following two probes: N25 (D22S75 locus; Oncor) and sc11.1 (Lindsay et al. 1993, 1995). The N25 probe is commonly used to detect hemizygosity of 22q11 in VCFS/DGS patients. The sc11.1 probe was derived from cosmid sc11.1 (Lindsay et al. 1993). In normal cells, the sc11.1 probe yields two hybridization signals on each chromosome 22 because of an apparent duplication in the region (Halford et al. 1993). The two signals, designated "11.1A" and "11.1B," are ~1.5 million bp apart (Lindsay et al. 1993). The position of these loci within the physical map of 22q11 is shown in figure 1*A*. Most VCFS and DGS patients are deleted for both loci (Lindsay et al. 1995). Results of FISH analysis of all the 26 patients are summarized in figure 2. In this group, 24/26 patients were hemizygous for the FISH probes. Two patients, BM26 and BM102, had hybridization signals on both copies of chromosome 22, suggesting that they did not have a 22q11 deletion.

STRP Analysis of the VCFS Patients

For the 14 polymorphic STRP markers, each of 26 patients and their unaffected family members were typed to further define the deletions of 22q11. The results of the marker analysis for the 26 VCFS patients are presented in figure 2. A single allele for a genetic marker is represented as an open box indicating homozygosity or hemizygosity at that locus. The shaded boxes indicate heterozygosity at a given locus. The group of VCFS patients, as a whole, had significantly lower levels of heterozygosity for markers D22S1638, D22S941, D22S1648, D22S944, D22S1623, D22S264, and D22S311 than did their unaffected relatives (table 2). These results suggest that all or part of the region encompassed by these markers is deleted among VCFS patients. The seven markers encompass a region of ~3 Mb in length.

Haplotype Analysis of Families

To define the proximal and distal boundaries of the deletion in each patient precisely, the genotype of the patients with respect to the 14 STRP markers was compared to both unaffected parents if available. Both unaffected parents were available for 15 cases, and only the mother was available for an additional 5 cases. We studied all 20 families. Representative results of this analysis are illustrated in figure 3. For example, the father of VCFS patient BM214, referred to as "BM216," carried alleles 6 and 4 for D22S1638, and BM215, the mother of BM214, carried alleles 1 and 6 at this locus. The child had only allele 4 contributed by the father but had no contribution from the mother. The relative orientation of D22S306 and D33S308 has not been established. BM214 was uninformative for D22S306, as indicated by the open box in the figure, but was heterozygous for D22S308. This type of deductive strategy was used to determine the parental origin of the deletion and the boundaries of the deletion with respect to the polymorphic markers. The haplotype analysis of the patients AP1136, BM58, BM102, and BM26 is also shown in figure 3. The summary of results from haplotype analysis of the 26 patients is shown in figure 1*B*. We found that 17 of the patients (AP1079, BM72, BM148, BM69, BM122, BM210, BM214, VCF1, AP1018, BM50, AP1088, BM78, BM171, AP1035, BM87, AP1136, and BM270; table 1) had the commonly occurring 3-Mb deletion that was flanked by the STRP markers D22S427 and D22S306/308. Haplotype analysis could not be performed on six VCFS patients (BM65, BM128, AP1012, BM17, BM139, and BM90) because their parents were not available for analysis. Results from FISH and genetic

Table 1

Clinical Findings in VCFS Patients

	Code No.	Age (years)/Sex	Physical Findings	Psychiatric Phenotype	Psychotic Symptoms
1	BM50	5/M	Face, SMCP, VSD	ADDH, cyclothymia	...
2	BM87	5/M	Face, SMCP, VSD, coronal craniosynostosis	Enuresis, encopresis	...
3	BM65	5/M	Face, SMCP, ASD, VSD, IAA, LW, scoliosis	No diagnosis	...
4	BM72	8/F	Face, SMCP, VSD, LW	ADHD, avoidant disorder	...
5	BM26	8/F	Face, SMCP, VSD, strabimus	ADD, avoidant disorder	...
6	AP1088	10/F	Face, CP, LW	Cyclothymia, ADHD, separation anxiety disorder	...
7	VCF-1	11/F	Face, SMCP, IAA	Bipolar II (rapid-cycler)	...
8	BM148	12/F	Face, OSMCP, HC, HP, ostepenia, Sprengel deformity	Bipolar II	...
9	BM210	12/F	Face, SMCP	Bipolar II, ADHD	...
10	BM58	13/M	Face, SMCP	Cyclothymia, ADHD	...
11	BM90	14/M	Face, unilateral cleft lip/cleft palate	Bipolar II (rapid-cycler), ADHD, OCD, enuresis, separation anxiety disorder	...
12	BM128	15/F	Face, OSMCP, TOF, HC, SNHL, TRV, thrombocytopenia	Bipolar II (rapid-cycler)	...
13	BM69	15/F	Face, SMCP, VSD	Bipolar II	...
14	BM139	16/F	Face, SMCP	ADHD	...
15	AP1136	16/M	Face, SMCP, ASD	Bipolar II, OCD	...
16	BM102	17/F	Mild face, CP, short stature	Bipolar II (rapid-cycler), ADHD	...
17	BM17	17/M	Face, SMCP, PDA, strabismus, hypospadias	Dysthymic disorder, avoidant disorder	...
18	BM78	17/M	Face, SMCP, VSD, LD	Bipolar I (rapid-cycler)	...
19	BM214	17/M	Face, OSMCP, HC, neutropenia, thrombocytopenia, strabismus	Dysthymia, ADD	...
20	BM270	18/M	Face, SMCP, seizures, scoliosis, dysarthria, TOF, VSD, PDA, BPV	ADD, major depression	...
21	BM122	20/M	Face, SMCP, SAS, syndactyly	Bipolar I	Paranoid and grandiose delusions
22	AP1018	22/M	Face, SMCP, VSD, thrombocytopenia	Bipolar I (rapid-cycler), OCD	Paranoid and grandiose delusions
23	AP1035	22/F	Face, CP, VSD, URIs, scoliosis	Bipolar II	...
24	BM171	25/F	Face, SMCP, LW	Bipolar II (rapid-cycler, drug induced)	...
25	AP1079	29/F	Face, OSMCP, VSD, rt AA, microcephaly	Schizoaffective-manic	Paranoid and grandiose delusions
26	AP1012	34/M	Face, SMCP, thrombocytopenia, sinus/ear infections	Schizoaffective-manic, simple phobia	Paranoid and grandiose delusions

NOTE.—A total of 26 VCFS patients, listed in code name, were assessed for physical as well as psychiatric findings. Face = mild facial dysmorphology consistent with VCFS; SMCP = submucous cleft palate; VSD = ventricular septal defect; IAA = interrupted aortic arch; LW = laryngeal web; HC = hypocalcemia; TOF = tetralogy of Fallot; SNHL = sensory neural hearing loss; TRV = tortuous retinal vessels; ASD = atrial septal defect; PDA = patent ductus arteriosis; BPV = bicuspid pulmonary valve; SAS = supravalvular aortic stenosis; CP = cleft palate; URI = upper respiratory tract infections; OSMCP = overt submucous cleft palate; and rt AA = right aortic arch.

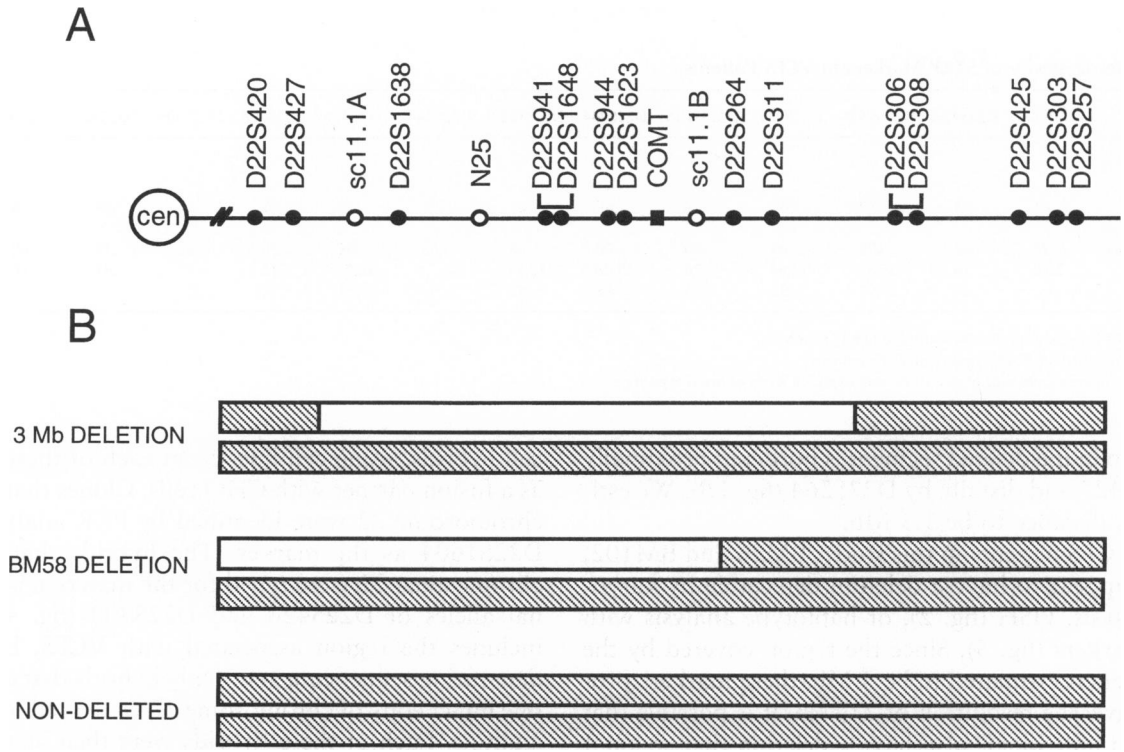

Figure 1 *A*, Order of the FISH (open circles), COMT gene (square), and STRP markers (filled circles). Markers whose order was not known were identified by brackets. *B*, Summary of haplotype analysis for 26 VCFS patients. The hatched boxes indicate the two copies of chromosome 22. The open box represents a region that shows a loss of heterozygosity.

markers analysis of this subset of patients were consistent with the presence with the common 3-Mb deletion (fig. 2). Among the patients who had the large common deletion, the proximal breakpoint was between markers D22S427 and D22S1638.

BM58 was unique among the patients tested in that he carried an unbalanced translocation t(18;22) and was hemizygous for all of 22pter-22q11 to a location somewhere between D22S1623 and D22S264 (figs. 1*B* and 3). Among this group of 18 patients, the smallest region

	PATIENT	D22S420	D22S427	D22S1638	D22S941	D22S1648	D22S944	D22S1623	D22S264	D22S311	D22S306	D22S308	D22S425	D22S303	D22S257	FISH RESULTS
1	AP 1079															del sc11.1A/B
2	BM 270															del N25
3	BM 72															del N25
4	BM 148															del N25
5	BM 65															del sc11.1A/B & N25
6	BM 69															del sc11.1A/B & N25
7	BM 128															del N25
8	BM 122															del N25
9	BM 210															del N25
10	BM 214															del N25
11	Vcf 1															del sc11.1A/B & N25
12	AP 1012															del sc11.1A/B
13	AP 1018															del sc11.1A/B & N25
14	BM 50															del sc11.1A/B
15	AP 1088															del sc11.1A/B & N25
16	BM 17															del F5
17	BM 78															del N25
18	BM 139															del N25
19	BM 171															del N25
20	AP 1035															del sc11.1A/B
21	BM 87															del N25
22	AP 1136															del sc11.1A/B
23	BM 90															del N25
24	BM 58															del N25
25	BM 26															not del sc11.1A/B or N25
26	BM 102															not del N25

Figure 2 Deletion analysis of VCFS patients. Polymorphic marker analysis using 14 STRP markers. Two alleles, denoted by the shaded boxes, indicate heterozygosity at a given locus; an unshaded box denotes that there is a single allele at the locus.

Table 2

Loss of Heterozygosity of STRP Markers in VCFS Patients

	D22S420	D22S427	D22S1638	D22S941	D22S1648	D22S944	D22S1623	D22S264	D22S311	D22S306	D22S308	D22S425	D22S303	D22S257
Heterozygosity in unaffected relatives[a]	.61	.68	.89	.63	.26	.66	.61	.79	.79	.55	.51	.68	.76	. 57
Heterozygosity in VCFS patients[b]	.85	.62	.08	.04	.00	.00	.08	.08	.08	.50	.62	.58	.69	.60
χ^2 [c]	2.38	.16	19.31	14.44	6.76	17.16	12.11	16.73	16.73	.12	.57	.41	.16	.002
P	...	...	>.95	>.95	>.95	>.95	>.95	>.95	>.95	...	...	...	...	...

[a] Frequency of heterozygosity in 38 unaffected family members.
[b] Observed frequency of heterozygosity in VCFS patients.
[c] Used to determine significance of deviation from expected levels of heterozygosity.

of overlap in the deleted segment is bordered proximally by D22S427 and distally by D22S264 (fig. 1*B*). We estimate this distance to be 1.5 Mb.

Two VCFS patients in this study, BM26 and BM102, did not appear to have a deletion detectable by karyotype analysis, FISH (fig. 2), or haplotype analysis with STRP markers (fig. 3). Since the region covered by the 14 markers is estimated to be 3 Mb, these markers provide an average resolution of 214 kb. It is possible that these two individuals also have a deletion that was not detectable by the methods we used. To determine definitively whether either of these patients have a deletion, we separated the two homologues of chromosome 22 and typed them with a large number of STS markers that were mapped to the 1.5-Mb region flanked by D22S427 and D22S264.

Somatic Hybrid Analysis of BM26 and BM102

To ascertain whether patients BM26 and BM102 carry a smaller deletion, we prepared a set of somatic cell hybrids, using cell lines from each of these patients as a fusion partner with CHO cells. Clones that retained chromosome 22 were identified by PCR analysis using D22S1604 as the marker. The hybrids that retained chromosome 22 were typed for the maternal and paternal alleles of D22S420 and D22S303 (fig. 4), which includes the region associated with VCFS, by use of denaturing sequencing gels. Only hybrids that contained one intact copy of chromosome 22 were used for further analysis. Each of these hybrids were then analyzed for the presence or absence of 71 STS markers (Morrow et al. 1995; B. Morrow and C. Carlson, unpublished data), which include the region covered by markers D22S427 and D22S264, an interval of ~1.5 Mb (Morrow et al. 1995). For BM26, we examined a total of five hybrids, four containing the maternal chromosome 22 and the other containing the paternal chromosome 22. For BM102, we examined two hybrids, each containing a different parental chromosome 22. In all of these hybrids, all of the markers tested were positive (data not

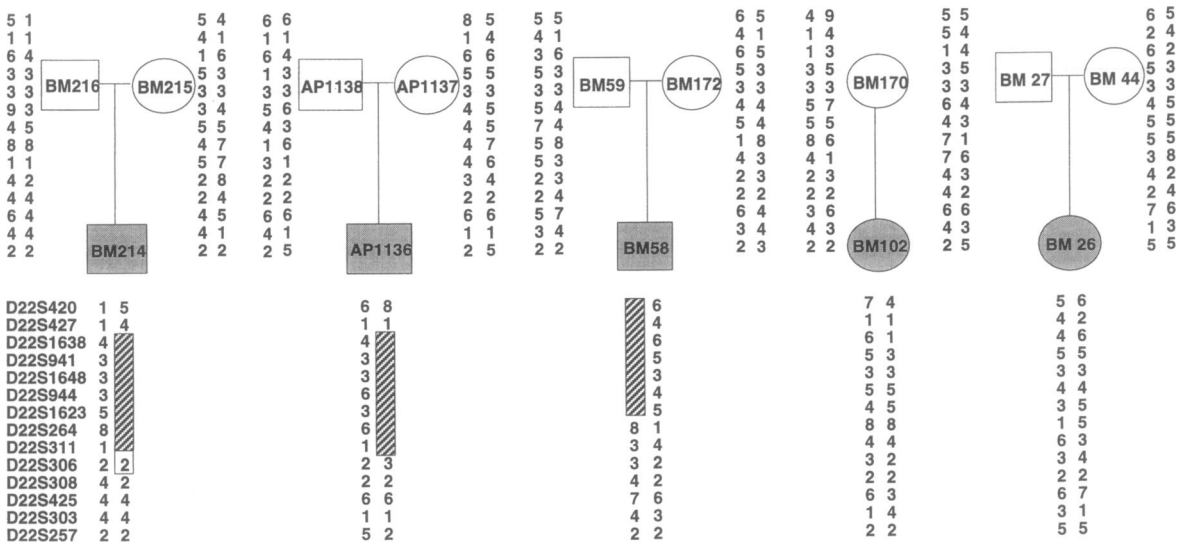

Figure 3 Genotype analysis of VCFS patients. Analysis for patient BM214, AP1136, BM58, BM102, and BM26. The haplotypes at the 14 polymorphic loci were deduced for this patients with unaffected parents. The hatched box represents loss of heterozygosity.

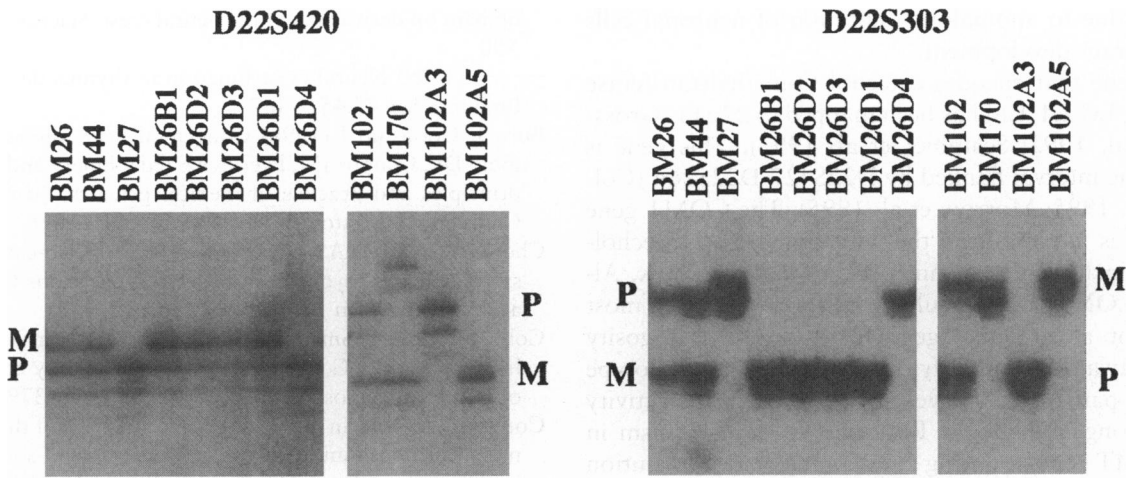

Figure 4 Identification of the maternal and paternal chromosome 22 in somatic cell hybrids. Typing with D22S420 and with D22S303. BM44 and BM27 are parents of BM26. BM170 is the mother of BM102. M = maternal allele; P = paternal allele.

shown). Since the 71 markers tested covered a region of 1.5 Mb, the marker distance is, on average, 21 kb. On the basis of these results, we conclude that, if BM26 and BM102 have deletions, they are, on average, <21 kb. The actual spacing between each of the individual markers may vary in physical distance; the 21 kb is an estimation based on the average.

Discussion

The goal of this study was to determine whether hemizygosity of a region within 22q11 could be associated with the psychiatric phenotypes observed in VCFS patients. We also wished to ascertain whether this region is the same as that associated with the physical anomalies in VCFS patients. We examined 26 VCFS patients (5–34 years of age) for psychiatric illness, using well-established criteria, and for 22q11 deletions, using 14 ordered STRP markers. Each of the patients were chosen randomly for the study as they returned to the Center for Craniofacial Disorders at the Montefiore Medical Center for annual clinical examinations. However, four of the adult patients who were part of this study had previous psychiatric evaluations (Pulver et al. 1994). It should be noted that patients with mild or no psychiatric symptoms were less likely to participate in the study. A large proportion of the patients affected with VCFS had a range of diagnoses that included ADHD and bipolar spectrum disorders. Most of the adult patients in this study were found to have bipolar spectrum disorders, including bipolar I and II disorders and schizoaffective-manic disorder. Psychotic symptoms with paranoid and grandiose delusions occurred commonly in the adult patients.

One of the most significant observations from this study is that two patients who were diagnosed to have VCFS and psychiatric disorders did not have a detectable deletion in 22q11. We have elsewhere described six VCFS patients who were diagnosed to have psychiatric disorders (Karayiorgou et al. 1995). We have genotyped each of these patients with the 14 polymorphic markers. Five of them had findings consistent with a 3-Mb deletion, and one had a 1.5-Mb deletion nested within the 3-Mb region. The 1.5-Mb region, flanked by D22S427 and D22S264, contains the critical region for the physical anomalies of VCFS (Morrow et al. 1995). Although the precise molecular basis for the etiology of VCFS is not known, the fact that no correlation can be made between the extent of 22q11 deletions and that nondeletion patients exhibit psychiatric anomalies suggests that the psychiatric and nonpsychiatric diagnoses in VCFS patients may have the same genetic etiology. Since VCFS is considered to be a developmental disorder, our results suggest that the psychiatric disorders observed in VCFS patients may also have their origins in early embryonic development.

During embryonic development, neural crest cells migrate into, and participate directly in the formation of, the pharyngeal arches. The structures derived from the pharyngeal arches include the face, neck, great blood vessels, concotruncal region of the heart, thymus, and parathyroid glands. These are among the tissues and organs affected in VCFS and DGS. The importance of neural crest cells in the development of these structures was demonstrated in animal models by neural crest ablation studies (Kirby et al. 1983; Bockman and Kirby 1984; Kirby and Bockman 1984) and by chick-quail chimera studies (Le Douarin 1980; Couly and Le Douarin 1988). A common pathway may exist that results in defects of the migration of neural crest cells into the pharyngeal arches as well as neurodevelopmental defects

perhaps due to anomalous migration of neuronal cells during brain development.

The gene that encodes catechol O-methyltransferase (COMT; EC 2.1.1.6) has been mapped to 22q11 (Grossman et al. 1992; Scambler et al. 1992). This gene is within the interval flanked by D22S427-D22S264 (Collins et al. 1995; Morrow et al. 1995). The COMT gene product is involved in the metabolism of catecholamines, including dopaminergic neurotransmitters. Although COMT, which is ubiquitously expressed, is most likely not a candidate gene for VCFS, hemizygosity could play a role in modifying the psychiatric phenotype in VCFS patients. The levels of COMT enzyme activity vary among individuals. Recently, a polymorphism in the COMT gene, resulting in an amino acid substitution from a valine at position 158 to a methionine, has been found. This substitution results in a three- to fourfold reduction in enzyme activity and is responsible for the variability in enzyme activity (Lachman et al. 1996). There was no association between the low/high activity allele among the normal population (Lachman et al. 1996), unaffected VCFS family members (Lachman et al. 1996), and the general schizophrenia population (Daniels et al. 1996). Preliminary results following the examination of this polymorphism in these 26 VCFS patients revealed an association between the met-containing allele and the development of the rapid-cycling form of bipolar disorder (Lachman et al. 1996). These results suggest that different alleles of COMT, and perhaps other deleted genes, may modify the psychiatric phenotype in VCFS patients.

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