A Quantitative MRI Study of Posterior Fossa Development in Velocardiofacial Syndrome

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Background: *Velocardiofacial syndrome (VCFS) has been identified as a risk factor for developing schizophrenia. Qualitative neuroimaging studies indicated that VCFS was frequently associated with abnormal development of structures in the posterior fossa of the brain. The objective of this investigation was to identify the specific structures affected in the posterior fossa and investigate the association of these neuroanatomic variations with behaviors potentially related to later-onset psychiatric disorders.*

Methods: *Twenty-four children and adolescents with VCFS individually matched for age and gender with 24 control subjects received magnetic resonance imaging scans. Analysis of covariance models were used to investigate regional brain differences. Association between brain areas and behaviors measured on the Child Behavior Checklist (CBCL) were assessed using simple regression models.*

Results: *Children with VCFS had significantly smaller size of vermal lobules VI–VII and the pons after adjusting for overall brain size. There were no significant associations between scores on the CBCL and measures of neuroanatomic variation within the VCFS group.*

Conclusions: *Structural alterations of the posterior fossa in VCFS are specifically limited to cerebellar vermis lobules VI–VII and pons. Previous literature has suggested that the vermis is involved in social cognition, and alteration of lobules VI–VII could therefore partially explain the neurobehavioral profile associated with VCFS.*
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Key Words: Velocardiofacial, VCFS, 22q11.2, brain, MRI

Introduction

Velocardiofacial syndrome (VCFS) is a congenital, autosomal dominant condition affecting approximately 1 out of 4000 live births (Tezenas Du Montcel et al 1996). Hallmark features of VCFS include cleft palate or velopharyngal insufficiency, cardiac abnormalities, a specific set of facial features, and learning disabilities (Goldberg et al 1993; Ryan et al 1997).

In the majority of diagnosed cases (~85%), the syndrome is due to a 3MB deletion on 22q11.2 (Carlson et al 1997; Shaikh et al 2000). At least 30 genes are encoded in the deleted segment. Among these, a few are highly expressed in brain tissue and are probably necessary for normal brain development (Funke et al 1997; Roberts et al 1997; Yamagishi et al 1999). From these genes, GSCL (for Goosecoide-like) seems a likely candidate for having significant influence on neurodevelopment in VCFS, as it is most strongly expressed in the pons and dorsal thalamus. This pattern of expression could be related to volumetric decrease of the inferior-posterior regions of the brain that have been reported in previous brain imaging experiments (Chow et al 1999; Devriendt et al 1996; Lynch et al 1995; Mitnick et al 1994). These qualitative brain imaging studies in VCFS suggest specific reductions in the size of the posterior fossa (Chow et al 1999; Devriendt et al 1996; Lynch et al 1995; Mitnick et al 1994), which have been confirmed by a more recent quantitative morphometric magnetic resonance imaging (MRI) brain study (Eliez et al 2000) that showed significant decreases in the cerebellar tissue volumes of children and adolescents with VCFS. This quantitative study, however, reported only cerebellar hemispheric volumes and did not investigate the midline cerebellar vermis, a region that has been previously reported as qualitatively decreased in this condition (Chow et al 1999; Mitnick et al 1994).

The elucidation of neuroanatomic variation in VCFS might help to clarify the nature and etiology of the serious cognitive and behavioral abnormalities associated with the syndrome. For example, it is highly likely that adults (Bassett and Chow 1999; Gothelf et al 1999; Murphy et al 1999) and children (Nicolson et al 1999; Usiskin et al 1999) with VCFS are at increased risk to develop psychosis and schizophrenia. In a recent prospective study of 50 adults with a deletion 22q11.2, Murphy et al (1999) demonstrated that 21 individuals (42%) had a history of

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major psychiatric disorder and, of these, 15 (30% of the total sample) had a history of psychosis. These adult psychiatric disorders might have earlier psycho-pathologic expressions during childhood and adolescence. Supporting this hypothesis are observations (Swillen et al 1997, 1999) of early behavioral problems objectified by scores in the clinical range for the following subscales of the Child Behavior Checklist (CBLC): social problems, withdrawn, attention problems, and thought problems.

In the study discussed here, we examined the neuroanatomy of the brainstem (including the pons) and cerebellar vermis, two anatomic regions within the posterior fossa that we predicted would be altered in VCFS. We then investigated the potential relation between specific morphologic changes of these regions and behavioral measures that have been demonstrated to be of value in defining the VCFS phenotype in previous studies (Swillen et al 1997, 1999).

Methods and Materials

Subjects

After providing a complete description of the study to the persons with VCFS and their parents, written informed consent was obtained under protocols approved by the Institutional Review Board of Stanford University. Only subjects with VCFS who proved to be deleted on chromosome 22q11.2 using a fluorescent in situ hybridization technique were included in the study. Children presenting the VCFS clinical phenotype without deletion were excluded to increase diagnostic certainty. Recruitment was performed through the Northern California VCFS association and by advertising on our web site (www-cap.stanford.edu). Brain volumetric results (total brain and lobar volumes) from a subset of these subjects have been previously reported (Eliez et al 2000). Normal comparison subjects, recruited through ads in newspaper and parent networks, were individually matched for age and gender, with a minimum intelligence quotient (IQ) of 85 (one SD below the mean). All subjects were in good health and without evidence of neurologic or psychiatric disorder.

The sample was composed of 8 female and 16 male subjects ($n = 24$) with a deletion 22q11.2, (mean age = 12.5 years, SD 4.0) and 24 normal control subjects (mean age = 12.7 years, SD 4.2). Four subjects with VCFS and five control subjects were 17 years of age or older.

Cognitive and Behavioral Measures

Standardized cognitive testing was administered to 47 subjects within 3 months of the scanning procedures. Each subject under 17 years of age received the Wechsler Intelligence Scale for Children-Revised (WISC-III; Wechsler 1991), and adolescents 17 years of age and older received the Wechsler Adult Intelligence Scale (WAIS; Wechsler 1997).

The CBCL 4-18 (Achenbach 1991) was used to obtain standardized ratings of behavioral problems in the group of children and adolescents with VCFS. Parents of each patient completed the questionnaire. The 112 items of the CBCL questionnaire yield three major composite scores, Total Behavioral Problems, Externalizing Behavioral Problems, and Internalizing Behavioral Problems. Scores on these three composites that exceed 63 ($T > 63$, 90th centile) are considered "clinically significant," whereas scores less than 60 ($T < 60$) are considered to be within the range of normality. Scores between 60 and 63 ($60 \leq T \leq 63$) are considered to be the borderline range. Additionally, profile scales or subscores are generated that further subdivide the major composite scores. The internalizing subscore is composed of withdrawn, somatic complaints, and anxious/depressed profiles. The externalizing subscore is comprised of delinquent and aggressive behavior profiles. Social, thought, and attention problem profiles also are generated. Scores on the profile scores that exceed 70 ($T > 70$, 98th centile) are considered "clinicaly significant," whereas scores less than 67 ($T < 67$) are considered to be within the range of normality. Scores between 67 and 70 ($67 \leq T \leq 70$) are considered to be the borderline range.

MRI Protocol

Magnetic resonance images of each subject's brain were acquired with a GE-Signa 1.5 T scanner (General Electric, Milwaukee, WI). Coronal images were acquired with a three-dimensional volumetric radio frequency spoiled gradient echo (SPGR) using the following scan parameters: repetition time = 35 msec, echo time = 6 msec, flip angle = 45°, number of excitation (NEX) = 1, matrix size = 256 × 192, field of view = 24 cm^2, slice thickness = 1.5 mm, 124 slices.

Image Processing and Measurement

The SPGR image data were imported into the program BrainImage 3.1T for semi-automated image processing analysis and quantification (Kates et al 1999). These procedures have been described and validated elsewhere (Kaplan et al 1997; Reiss et al 1998; Subramaniam et al 1997).

Circumscription and measurement of the vermis, brainstem, and their component parts was based on a previously existing protocol (Mostofsky et al 1998a, 1998b). This protocol consists of first determining the best midsagittal slice based on clarity of the cerebellar vermis, cerebral aqueduct, corpus callosum, and spinal cord (Figure 1). The midsagittal slice was generated by rotating the brain in the x, y, and z planes, treating the brain as a three-dimensional object.

The vermis was then divided into three regions of interest (ROIs) by circumscribing lobules I–V, VI–VII, and VIII–X separately, following all fissures that exceeded 2 pixels in width. Interrater reliability for these structures was calculated using a repeated measures interclass correlation coefficient. These values were .93 for lobules I–V, .95 for lobules VI–VII, and .96 for lobules VIII–X.

To reliably measure the brainstem, three perpendicular lines were drawn that defined the midbrain–pons, the pons–medulla, and the medulla–spinal cord borders. These lines were automatically generated by BrainImage when given five neuroanatomic

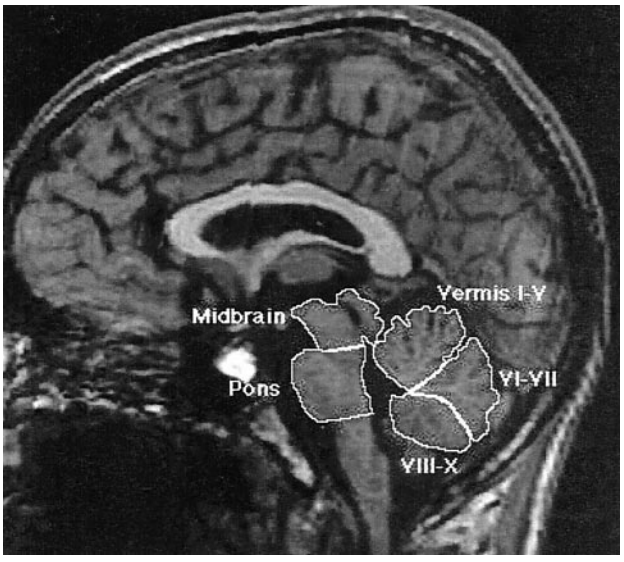

Figure 1. Circumscription of the midbrain, pons, and cerebellar vermis on the midsagittal image. The vermis was divided into three portions: the anterior vermis (lobules I–V), lobules VI–VII, and lobules VIII–X.

landmarks (superior and inferior pontine notches, the endpoints of the brainstem, and the tip of the foramen magnum). Interrater reliability values for the midbrain, pons, and forth ventricle were .88, .91, and .94, respectively.

In addition, because the pons is a structure of particular interest, a protocol was designed to obtain volumetric measurements. On the midsagittal slice, the superior border of the pons was defined by drawing a straight line from the superior pontine notch perpendicular to the center line axis (Figure 1). The inferior border was defined similarly using the inferior pontine notch. The lateral border of the pons was defined as the most lateral slice (in a sagittal view) where the pons and crus cerebri of the midbrain were connected.

Statistical Analyses

Data were first examined for normality to conform to the assumptions of the parametric statistics employed, and Levene's test was run on all the variables to ensure that the assumption of equality of variance was met. Multiple analysis of covariance (MANCOVA) was utilized to determine if the VCFS and comparison group had unique patterns of morphologic brain changes for vermis subregions, midbrain and pons with total brain tissue as a covariate. Analyses of total brain tissue were performed with one-way analyses of variance (ANOVA) using diagnosis (VCFS vs. comparison subjects) as a between-subject factor. Analysis of covariance (ANCOVA) was used for subregion comparisons to more accurately quantify group differences after statistically adjusting for the effect of total brain volumes. Regression analyses were used with the VCFS group to test for predictive relationships between brain subregions, and IQ and CBCL scores. A p-value of .01 (two-tailed) was chosen as the significance threshold.

Results

Group Differences in Tissue Volumes

As shown in Table 1, the total brain tissue volume was approximately 12% smaller in the VCFS group relative to the control group [ANOVA, $F(1,46) = 23.9$, $p < .0001$]. Reduced tissue volume was comparable for the left and right hemispheres when analyzed separately.

To investigate the hypothesis that midsagittal brain development in VCFS differs from that observed in control subjects, a MANCOVA was computed with group as a main effect (VCFS vs. control) and total brain volume entered as a covariate to statistically control for differences in overall brain size. Dependent variables consisted of vermis I–V, VI–VII, VIII–X, midbrain, and pons area. The Wilks λ of .72 [$F(5,41) = 3.2$, $p = .01$] indicated a unique pattern of cerebral morphologic variation that distinguishes persons with VCFS from control subjects.

Follow-up analyses of variance were utilized to specify the regional area differences. When controlling for differences in total tissue volume, results indicated that midsagittal areas of vermal lobules VI–VII [$F(1,45) = 7.4$, $p < .01$] and the pons [$F(1,45) = 7.5$, $p < .01$] were signifi-

Table 1. Summary of Posterior Fossa Measurements

	Control group ($n = 24$)	VCFS group ($n = 24$)	p values	F values
Total brain volume	1295.9 ± 98	1137.7 ± 124	<.0001[a]	23.9
Vermis area				
I–V	5.13 ± 0.6	4.77 ± 0.7	ns[b]	0.04
VI–VII	3.74 ± 0.6	3.12 ± 0.8	<.01[b]	7.4
VIII–X	3.88 ± 0.7	3.21 ± 0.5	ns[b]	1.8
Midbrain area	3.09 ± 0.3	3.04 ± 0.2	ns[b]	0.6
Pons area	5.81 ± 0.5	5.03 ± 0.6	<.01[b]	7.5
Pons volume	13.82 ± 1.5	10.82 ± 1.3	<.0001[b]	21.1

Areas and volumes are measured in cm^2 and cm^3, respectively. VCFS, velocardiofacial syndrome.
[a]Analysis of variance.
[b]Analysis of covariance, with total brain volume as a covariate.

Table 2. Cognitive and Behavioral Measurements

	Control group (n = 23)	VCFS group (n = 24)	p value	F value
Full scale IQ	117.4 ± 11.7	69.5 ± 17.2	<.0001	123.1
Child Behavior Checklist				
Total T score		61.0 ± 9.3		
Internalizing T score		58.0 ± 10		
Withdrawn		60.5 ± 12.2		
Somatic		57.8 ± 6.4		
Anxious/depressed		57.3 ± 8.3		
Externalizing T score		53.1 ± 9.6		
Delinquent		54.5 ± 5.6		
Aggressive		55.8 ± 6.7		
Social problems		64.9 ± 7.7		
Thought problems		62.0 ± 9.7		
Attention problems		65.8 ± 9.0		

VCSF, velocardiofacial syndrome.

cantly decreased in subjects with VCFS. The volume of the pons was also significantly smaller in the VCFS population [$F(1,45) = 21.1$, $p < .0001$]. Group differences in vermal lobules I–V ($p = .8479$) and VIII–X ($p = .1885$), and midbrain ($p = .4195$) were nonsignificant.

Cognition and Behavior in VCFS

Results are presented in Table 2. Subjects' Full-scale IQ's (FSIQ) ranged from moderately retarded to normal. The mean FSIQ for subjects with VCFS was 69.5 ± 17.2. One subject's FSIQ was above 100, and 13% ($n = 3$) were in the normal range (>85). Among these subjects with VCFS, 33% ($n = 8$) were in the "borderline intelligence" range (71–84), and 54% ($n = 13$) were in the retarded range (≤70). Twenty-nine percent ($n = 7$) were moderately retarded (40–55), and 25% ($n = 6$) were mildly retarded (55–70). No subjects with VCFS were severely retarded. The mean FSIQ for control subjects was 117.4 ± 11.7.

On the CBCL (Table 2), the "Attention Problems" (mean = 65.8), "Social Problems" (mean = 64.9), "Thought Problems" (mean = 62.0), and "Withdrawn (behavior)" (mean = 60.5) scales had the highest means, respectively. Although none of these scales' means reached the borderline clinical range (67–69), 38% of the sample was in the borderline or clinically significant range on each of the "Attention Problems," "Social Problems," and "Thought Problems" scales and 21% on the "Withdrawn" scale. In terms of the summary scale scores, the mean score (m) on the "Externalizing" scale ($m = 53.1$) and the "Internalizing" scale ($m = 58.0$) were in the normal range, and the "Total Problems" scale ($m = 61.0$) was in the borderline clinical range. Thirty-three percent of the sample was in the borderline or clinically significant range on the "Externalizing" scale, 42% on the "Internalizing" scale, and 67% on the "Total Problems" scale.

Neuroanatomy, IQ, and Behavior Associations

For the 23 control subjects and 24 subjects with VCFS, there was a statistically significant group difference ($F = 123.1$, $p < .0001$) in mean full scale IQ scores (Table 2). The goal of our analyses was to compare the influence of total and regional brain volumes on IQ on typically developing children and the VCFS population. Results indicated no relationship between cerebral volume and IQ in either group ($R^2 = .001$, $p = .87$ and $R^2 = .05$, $p = .29$ for control and VCFS group, respectively). To examine whether brain subregions (vermis, pons, midbrain) were predictive of IQ scores, we utilized simple regression procedures. None of the subregional brain areas contributed significantly to the prediction of IQ scores in controls or subjects with VCFS.

Associations between four CBCL subtests (Attention, Social, Thought, and Withdrawn) and brain structures were investigated. The choice of the four CBCL subtests to include in these analyses was based on previous studies of children and adolescents with VCFS (Attention, Social and Withdrawn) (Swillen et al 1997) as well as potential behavioral symptoms of childhood-onset schizophrenia (Thought Problems). To examine whether significantly decreased brain subregions (vermis VI–VII and pons) were predictive of CBCL scores, we utilized simple regression procedures. Because there was no correlation between total brain volume and CBCL scores, we did not statistically control for cerebral volume. None of the subregional brain areas contributed significantly to the prediction of CBCL subscores. The strongest association

was observed between vermis VI–VII area and Thought subscore ($F = 4.5$, $R^2 = .17$, $p = .05$).

Discussion

Our data suggest that the pons and vermal lobules VI–VII are disproportionally reduced in VCFS. Because overall brain tissue was taken into consideration in these analyses, it appears that the vermis and the pons are particularly affected in VCFS, beyond the significant global tissue reduction that also was observed. Both the findings of decreased vermis and decreased overall brain tissue size in VCFS help to support our previous quantitative neuroimaging study on VCFS, which reported an 11% smaller overall brain tissue volume (Eliez et al 2000). Specifically, a significantly smaller cerebellar volume in VCFS was found when compared to a normal control group even after adjusting for differences in total brain volumes. Though our studies are currently the only quantitative investigations of VCFS neuroanatomy, other qualitative studies also have reported brain tissue decreases and aberrant cerebellar morphology (Altman et al 1995; Devriendt et al 1996; Lynch et al 1995; Mitnick et al 1994).

The pons was found to be significantly reduced in size when using both volumetric and midsagittal area techniques. This finding is particularly interesting given the emerging genetic knowledge on VCFS. In particular, GSCL, a gene in the 22q11 region, may be responsible for the observed abnormal development of the pons and vermis. Gene GSCL is expressed early during embryogenesis in a limited number of tissues and has homeobox gene structure (Gottlieb et al 1997). The DNA sequence-specific recognition sites of this gene suggest a transcription regulation function, including its own transcription autoregulation. The mouse homologue of GSCL is expressed in the anterior portion of the embryo and, in the brain, is most expressed in the pons and dorsal thalamus (Gottlieb et al 1998). In the adult mouse brain, no expression was observed in the pons, but expression was seen in the interpeduncular nucleus located in the ventral midbrain. This led Gottlieb (Gottlieb et al 1998) to suspect a consequent deficiency of the limbic system in VCFS that could result in abnormal regulation of arousal, abnormal response to stress, and avoidant behavior.

Developmentally, the pons and the cerebellum share a common cellular ancestry. During embryogenesis, the pons and the cerebellum arise primarily from the metencephalon, the most anterior region of the hindbrain (rombencephalon), developmentally distinct from both the midbrain and the medulla (Moore and Persaud 1993; Rowitch et al 1999). Because GSCL is expressed early in development of the posterior fossa, absence or reduction of its protein product during a critical time period could result in abnormal development of both the pons and cerebellum. Interestingly, the cerebellar hemispheres and posterior vermis are predominantly derived from metencephalic tissue, whereas the tissue of the anterior vermis, like the midbrain, is of mesencephalic origin (Hallonet et al 1990; Martinez and Alvarado-Mallart 1989; Millet et al 1996; Rowitch et al 1999). This may explain why no significant differences were found in volumes of the anterior vermis of subjects with VCFS.

In addition, the pons and the cerebellum are cytoarchitecturally linked. The basal pontine nuclei project major afferent pathways to the cerebellar cortex (Yachnis and Rorke 1999). These projections are in the form of mossy fibers from the basis pontis, which synapse on Purkinje cells early in development (Grishkat and Eisenman 1995; Yachnis and Rorke 1999). Complimentarily, all of the neurons in the basis pontis project exclusively to the cerebellum; the corticopontocerebellar pathway is the primary method of communication between cerebrum and cerebellum (Schmahmann 1991). Because a lack of adequate stimulation can cause neuron death during development (Cabelli et al 1995; LeVay et al 1978), it is possible that damage to the pons may in turn result in degeneration of cerebellar tissue.

Velocardiofacial syndrome is not the first neurogenetic syndrome to reportedly have an aberrant posterior fossa and cerebellar vermal size. Specifically, Joubert syndrome and fragile X both have been shown to have decreased vermal areas (Guerreiro et al 1998; Holroyd et al 1991; Mostofsky et al 1998a). Subjects with both Joubert syndrome and fragile X also typically possess social and communication problems resembling autistic behavior (Cohen et al 1988, 1991; Holroyd et al 1991). Conversely, subjects with Williams syndrome, who are unusually socially outgoing and overly friendly (Jones et al 2000), show significant increases in the posterior vermis and the neocerebellar hemispheres relative to normal controls (Jernigan and Bellugi 1990; Wang et al 1992). Thus, there appears to be a possible relationship between posterior vermis size and level of social drive as evidenced by comparing neurogenetic disorders with prominent affective components.

These observations are concordant with the description of specific social difficulties in VCFS. All the studies reporting observation of social behavior point to poor social interactions, shyness, behavioral inhibition, and withdrawal (Gerdes et al 1999; Golding-Kushner et al 1985; Heineman-de Boer et al 1999; Swillen et al 1997, 1999). Social and communication problems described in the samples of children with VCFS could be a prelude to psychiatric disorders like schizophrenia (Nicolson and Rapoport 2000).

We did not demonstrate significant associations be-

tween reduced vermis size and behavioral problems as measured with the CBCL. There are at least three possible explanations for this observation. First, the number of subjects described in this study might not be large enough to detect an association between these specific behavioral problems and alteration of vermis. Second, the problems observed on the subscales of the CBCL might be related to another area or network of the brain not measured in our study. Third, if the vermis is involved in social cognition or thought processes, the CBCL might not be the most appropriate instrument to document communication and social deficits, or to identify thought disorder in children. Cognitive tests more sensitive to internalizing problems, designed specifically to measure social drive and avoidance behaviors may be required to see significant correlations between specific brain regions and these cognitive and behavioral features. Similarly, we could not detect any association between total brain volume and IQ in the VCFS or control group. Although still controversial, previous studies with relatively large sample size (Pennington et al 2000; Reiss et al 1996) have indicated that total brain volume is positively correlated with IQ. The most likely explanation for the lack of this association in our study is, at least for the control group, limited sample size.

Velocardiofacial syndrome is a model system for examining the multifaceted nature of behavioral development, and therefore must be investigated from many perspectives. Future research should examine the neuroanatomic differences between subjects with VCFS who develop schizophrenia and those who do not to determine whether pons size is explicitly related to psychosis (Sachdev and Brodaty 1999) and not simply to VCFS. In addition, functional imaging will allow the visualization of neural activation resulting from behavioral and cognitive stimulation, and may provide further evidence of the role of the vermis in emotion and social drive.

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References

Achenbach TM (1991): *Manual for the Child Behavior Checklist/4-18 and 1991 Profile.* Burlington: University of Vermont, Department of Psychiatry.

Altman DH, Altman NR, Mitnick RJ, Shprintzen RJ (1995): Further delineation of brain anomalies in velo-cardio-facial syndrome. *Am J Med Genet* 60:174–175.

Bassett AS, Chow EW (1999): 22q11 deletion syndrome: A genetic subtype of schizophrenia. *Biol Psychiatry* 46:882–891.

Cabelli RJ, Hohn A, Shatz CJ (1995): Inhibition of ocular dominance column formation by infusion of NT-4/5 or BDNF. *Science* 267:1662–1666.

Carlson C, Papolos D, Pandita RK, et al (1997): Molecular analysis of velo-cardio-facial syndrome patients with psychiatric disorders. *Am J Hum Genet* 60:851–859.

Chow EW, Mikulis DJ, Zipursky RB, et al (1999): Qualitative MRI findings in adults with 22q11 deletion syndrome and schizophrenia. *Biol Psychiatry* 46:1436–1442.

Cohen IL, Fisch GS, Sudhalter V, et al (1988): Social gaze, social avoidance, and repetitive behavior in fragile X males: A controlled study. *Am J Ment Retard* 92:436–446.

Cohen IL, Vietze PM, Sudhalter V, et al (1991): Effects of age and communication level on eye contact in fragile X males and non-fragile X autistic males. *Am J Med Genet* 38:498–502.

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

Eliez S, Schmitt JE, White CD, Reiss AL (2000): Children and adolescents with velocardiofacial syndrome: A volumetric MRI study. *Am J Psychiatry* 157:409–415.

Funke B, Saint-Jore B, Puech A, et al (1997): Characterization and mutation analysis of goosecoid-like (GSCL), a homeodomain-containing gene that maps to the critical region for VCFS/DGS on 22q11. *Genomics* 46:364–372.

Gerdes M, Solot C, Wang PP, et al (1999): Cognitive and behavior profile of preschool children with chromosome 22q11.2 deletion. *Am J Med Genet* 85:127–133.

Goldberg R, Motzkin B, Marion R, et al (1993): Velo-cardio-facial syndrome: A review of 120 patients. *Am J Med Genet* 45:313–319.

Golding-Kushner KJ, Weller G, Shprintzen RJ (1985): Velo-cardio-facial syndrome: Language and psychological profiles. *J Craniofac Genet Dev Biol* 5:259–266.

Gothelf D, Frisch A, Munitz H, et al (1999): Clinical characteristics of schizophrenia associated with velo-cardio-facial syndrome. *Schizophr Res* 35:105–112.

Gottlieb S, Emanuel BS, Driscoll DA, et al (1997): The DiGeorge syndrome minimal critical region contains a goosecoid-like (GSCL) homeobox gene that is expressed early in human development. *Am J Hum Genet* 60:1194–1201.

Gottlieb S, Hanes SD, Golden JA, et al (1998): Goosecoid-like, a gene deleted in DiGeorge and velocardiofacial syndromes, recognizes DNA with a bicoid-like specificity and is expressed in the developing mouse brain. *Hum Mol Genet* 7:1497–1505.

Grishkat HL, Eisenman LM (1995): Development of the spinocerebellar projection in the prenatal mouse. *J Comp Neurol* 363:93–108.

Guerreiro MM, Camargo EE, Kato M, et al (1998): Fragile X syndrome. Clinical, electroencephalographic and neuroimaging characteristics. *Arq Neuropsiquiatr* 56:18–23.

Hallonet ME, Teillet MA, Le Douarin NM (1990): A new approach to the development of the cerebellum provided by the quail-chick marker system. *Development* 108:19–31.

Heineman-de Boer JA, Van Haelst MJ, Cordia-de Haan M,

Beemer FA (1999): Behavior problems and personality aspects of 40 children with velo-cardio-facial syndrome. *Genet Couns* 10:89–93.

Holroyd S, Reiss AL, Bryan RN (1991): Autistic features in Joubert syndrome: A genetic disorder with agenesis of the cerebellar vermis. *Biol Psychiatry* 29:287–294.

Jernigan TL, Bellugi U (1990): Anomalous brain morphology on magnetic resonance images in Williams syndrome and Down syndrome. *Arch Neurol* 47:529–533.

Jones W, Bellugi U, Lai Z, et al (2000): Hypersociability in Williams syndrome. *J Cogn Neurosci* 12(suppl 1):30–46.

Kaplan DM, Liu AM, Abrams MT, et al (1997): Application of an automated parcellation method to the analysis of pediatric brain volumes. *Psychiatry Res* 76:15–27.

Kates WR, Warsofsky I, Patwardhan A, et al (1999): Automated Talairach-based parcellation and measurement of cerebral lobes in children. *Psychiatry Res* 91:11–30.

LeVay S, Stryker MP, Shatz CJ (1978): Ocular dominance columns and their development in layer IV of the cat's visual cortex: A quantitative study. *J Comp Neurol* 179:223–244.

Lynch DR, McDonald-McGinn DM, Zackai EH, et al (1995): Cerebellar atrophy in a patient with velocardiofacial syndrome. *J Med Genet* 32:561–563.

Martinez S, Alvarado-Mallart R (1989): Rostral cerebellum originates from the caudal portion of the so-called "mesencephalic" vesicle: A study using chick/quail chimeras. *Eur J Neurosci* 1:549–560.

Millet S, Bloch-Gallego E, Simeone A, Alvarado-Mallart RM (1996): The caudal limit of Otx2 gene expression as a marker of the midbrain/hindbrain boundary: A study using in situ hybridisation and chick/quail homotopic grafts. *Development* 122:3785–3797.

Mitnick RJ, Bello JA, Shprintzen RJ (1994): Brain anomalies in velo-cardio-facial syndrome. *Am J Med Genet* 54:100–106.

Moore KL, Persaud TVN (1993): *The Developing Human: Clinically Oriented Embryology.* Philadelphia: Saunders.

Mostofsky SH, Mazzocco MM, Aakalu G, et al (1998a): Decreased cerebellar posterior vermis size in fragile X syndrome: Correlation with neurocognitive performance. *Neurology* 50:121–130.

Mostofsky SH, Reiss AL, Lockhart P, Denckla MB (1998b): Evaluation of cerebellar size in attention-deficit hyperactivity disorder. *J Child Neurol* 13:434–439.

Murphy KC, Jones LA, Owen MJ (1999): High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Arch Gen Psychiatry* 56:940–945.

Nicolson R, Giedd JN, Lenane M, et al (1999): Clinical and neurobiological correlates of cytogenetic abnormalities in childhood-onset schizophrenia. *Am J Psychiatry* 156:1575–1579.

Nicolson R, Rapoport JL (2000): Childhood-onset schizophrenia: What can it teach us? In: Rapoport JL, editor. *Childhood Onset of "Adult" Psychopathology.* Washington, DC: American Psychiatric Press, 167–192.

Pennington BF, Filipek PA, Lefly D, et al (2000): A twin MRI study of size variations in human brain. *J Cogn Neurosci* 12:223–232.

Reiss AL, Abrams MT, Singer HS, et al (1996): Brain development, gender and IQ in children. A volumetric imaging study. *Brain* 119:1763–1774.

Reiss AL, Hennessey JG, Rubin M, et al (1998): Reliability and validity of an algorithm for fuzzy tissue segmentation of MRI. *J Comput Assist Tomogr* 22:471–479.

Roberts C, Daw SC, Halford S, Scambler PJ (1997): Cloning and developmental expression analysis of chick Hira (Chira), a candidate gene for DiGeorge syndrome. *Hum Mol Genet* 6:237–245.

Rowitch DH, Danielian PS, McMahon AP, Zec N (1999): Cystic malformation of the posterior cerebellar vermis in transgenic mice that ectopically express Engrailed-1, a homeodomain transcription factor. *Teratology* 60:22–28.

Ryan AK, Goodship JA, Wilson DI, et al (1997): Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: A European collaborative study. *J Med Genet* 34:798–804.

Sachdev PS, Brodaty H (1999): Mid-sagittal anatomy in late-onset schizophrenia. *Psychol Med* 29:963–970.

Schmahmann JD (1991): An emerging concept. The cerebellar contribution to higher function. *Arch Neurol* 48:1178–1187.

Shaikh TH, Kurahashi H, Saitta SC, et al (2000): Chromosome 22-specific low copy repeats and the 22q11.2 deletion syndrome. Genomic organization and deletion endpoint analysis. *Hum Mol Genet* 9:489–501.

Subramaniam B, Hennessey JG, Rubin MA, et al (1997): Software and methods for quantitative imaging in neuroscience: The Kennedy Krieger Institute Human Brain Project. In: Koslow SH, Huerta MF, editors. *Neuroinformatics: An Overview of the Human Brain Project.* Mahwah, NJ: Erlbaum.

Swillen A, Devriendt K, Legius E, et al (1997): Intelligence and psychosocial adjustment in velocardiofacial syndrome: A study of 37 children and adolescents with VCFS. *J Med Genet* 34:453–458.

Swillen A, Devriendt K, Legius E, et al (1999): The behavioural phenotype in velo-cardio-facial syndrome (VCFS): From infancy to adolescence. *Genet Couns* 10:79–88.

Tezenas Du Montcel S, Mendizabai H, Ayme S, et al (1996): Prevalence of 22q11 microdeletion. *J Med Genet* 33:719.

Usiskin SI, Nicolson R, Krasnewich DM, et al (1999): Velocardiofacial syndrome in childhood-onset schizophrenia. *J Am Acad Child Adolesc Psychiatry* 38:1536–1543.

Wang PP, Hesselink JR, Jernigan TL, et al (1992): Specific neurobehavioral profile of Williams' syndrome is associated with neocerebellar hemispheric preservation. *Neurology* 42: 1999–2002.

Wechsler D (1991): *Wechsler Intelligence Scale for Children—Third Edition. Manual.* San Antonio: Psychological Corp.

Wechsler D (1997): *Wechsler Adult Intelligence Scale—Third Edition. Administration and Scoring Manual.* San Antonio: Psychological Corp.

Yachnis AT, Rorke LB (1999): Cerebellar and brainstem development: An overview in relation to Joubert syndrome. *J Child Neurol* 14:570–573.

Yamagishi H, Garg V, Matsuoka R, et al (1999): A molecular pathway revealing a genetic basis for human cardiac and craniofacial defects. *Science* 283:1158–1161.