

# Parental Origin of the Deletion 22q11.2 and Brain Development in Velocardiofacial Syndrome

## A Preliminary Study

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**Background:** As children with velocardiofacial syndrome (VCFS) develop, they are at increased risk for psychopathology; one third will eventually develop schizophrenia. Because VCFS and the concomitant symptomatology result from a known genetic origin, the biological and behavioral characteristics of the syndrome provide an optimal framework for conceptualizing the associations among genes, brain development, and behavior. The purpose of this study was to investigate the effect of the parental origin of the 22q11.2 microdeletion on the brain development of children and adolescents with VCFS.

**Methods:** Eighteen persons with VCFS and 18 normal control subjects were matched individually for age and sex. Results of DNA polymorphism analyses determined the parental origin of the deletion. Nine persons with VCFS had a deletion on the maternally derived chromosome 22; 9 persons, on the paternally derived chromosome 22. High-resolution magnetic resonance imag-

ing scans were analyzed to provide quantitative measures of gray and white matter brain tissue.

**Results:** Total brain volume was approximately 11% smaller in the VCFS group than in controls. Comparisons between VCFS subgroups (maternal vs paternal microdeletion 22q11.2) indicated a significant 9% volumetric difference in total volume of cerebral gray matter (volume was greater in patients with paternal microdeletion) but not cerebral white matter. Significant age-related changes in gray matter were detected for subjects whose 22q11.2 deletion was on the maternal chromosome.

**Conclusions:** Children and adolescents with VCFS experience major alterations in brain volumes. Significant reduction in gray matter development is attributable to presence of 22q11.2 microdeletion on the maternal chromosome.

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**V**ELOCARDIOFACIAL syndrome (VCFS) is a common genetic condition associated with physical features, including heart malformations, palatal abnormalities, and characteristic facial dysmorphism.<sup>1</sup> The high frequency of VCFS (1 in 2000-4000 live births)<sup>2</sup> ranks this condition as one of the most common genetic causes of learning disabilities and mild mental retardation. In most diagnosed cases, the syndrome is due to a 3 megabase (Mb) de novo deletion on chromosome 22q11.2,<sup>3</sup> and can occur on either parental chromosome. At least 30 genes are encoded in the common deleted segment.<sup>4</sup> Among these, a few are expressed in brain tissue, and some are likely to be essential for normal brain development.<sup>5-7</sup>

A relation between VCFS and schizophrenia has been suggested through numerous clinical research studies. After Shprintzen et al<sup>8</sup> published the first definition of VCFS, evidence from clinical studies demonstrated an increased prevalence of psychiatric disorders in the population

with VCFS.<sup>9-11</sup> The early investigations of VCFS and risk for psychopathology noted an elevated incidence of schizophrenia and schizoaffective disorders among adults with this genetic condition.<sup>9</sup> A subsequent investigation<sup>12</sup> in children and young adults with VCFS proposed an etiologic link with bipolar rather than schizophrenic disorders; 64% of subjects met the *DSM-III-R*<sup>13</sup> criteria for bipolar disorders, whereas only 6% had a diagnosis of schizoaffective disorder. More recent publications have pointed again toward a predisposition for schizophrenia.<sup>14-16</sup> At least 4 studies have demonstrated an overrepresentation of the 22q11.2 deletion in samples of persons with a diagnosis of schizophrenia; 2% to 6% of persons with schizophrenia in these samples had the 22q11.2 deletion.<sup>17-20</sup> Of 46 patients with childhood-onset schizophrenia, Nicolson and Rapoport<sup>19</sup> found that 6.4% had the 22q11.2 deletion.<sup>21</sup> Publications that address the VCFS-schizophrenia association generally concur that 25% to 30% of children with VCFS will eventually develop schizophrenia or psycho-

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## SUBJECTS AND METHODS

### SUBJECTS

Eighteen subjects (**Table 1**), 11 male and 7 female, with a mean ( $\pm$  SD) age of  $11.9 \pm 3.3$  years (range, 6.3-17.9 years) and a diagnosis of a 22q11.2 de novo microdeletion confirmed by fluorescent in situ hybridization (FISH) were included in the study. All subjects were identified as having the typical "large" 3 Mb deletion.<sup>28</sup> Recruitment was performed through the Northern California VCFS association and advertising on our Web site (<http://www-cap.stanford.edu>). 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, Stanford, Calif. A previous report on brain development in VCFS used a subsample of the subjects reported in this article.

Eighteen normal control subjects were matched for sex and age (each subject was individually matched within 14 months; mean [ $\pm$  SD] age,  $12.5 \pm 3.8$  years; range, 5.8-19.1 years). Controls were recruited through advertisement in local newspapers and parent groups' newsletters or among nonaffected siblings of children with identified genetic conditions (fragile X and Turner syndromes). A minimum IQ of 85 (1 SD below the population mean) and absence of previous neurologic or psychiatric disorder were used as an inclusion criteria for controls.

### GENETIC ANALYSIS

The deletions were verified and their extent was determined by means of 2-color FISH, with cosmid probes *DO832* (catechol-O-methyl transferase gene [*COMT*]) and *N48C12* (*D22S264*) as described.<sup>17</sup> The probes are specific for the proximal and distal deletion regions, respectively.<sup>29</sup> Parental origins of the deletions were established using DNA polymorphism analysis with standard techniques.<sup>17</sup> Subjects and their parents underwent genotyping for *D22S941*, *D22S944*, and *D22S264*, 3 polymorphic dinucleotide repeat markers located within the commonly deleted region. Nine subjects (3 female and 6 male) had deletions of maternal origin; 9 (4 female and 5 male), paternal origin.

### MAGNETIC RESONANCE IMAGING PROTOCOL, IMAGE PROCESSING, AND MEASUREMENT

Magnetic resonance images of each subject's brain were acquired using a 1.5-T scanner (GE-Signa; General Electric, Milwaukee, Wis). Coronal images were acquired using a 3D-volumetric radio frequency spoiled gradient echo (SPGR) pulse sequence with the following scan parameters: repetition time, 35 milliseconds; echo time, 6 milliseconds; flip angle, 45°; number of excitation, 1; matrix size,  $256 \times 192$  pixels; field of view, 24 cm<sup>2</sup>; slice thickness, 1.5 mm; 124 slices. The SPGR image data were imported into a publicly available program (BrainImage; A.L.R., Stanford [available at: <http://www-cap.stanford.edu/research/neuroimaging/imageanalysis/brainimage.html>]) for semiautomated image-processing analysis and quantification. In summary, the image-processing steps are (1) correction of voxel intensity nonuniformity (secondary to inhomogeneity of the radio-frequency field); (2) removal of nonbrain tissues such as scalp, skull, and vasculature; (3) segmentation of the brain into constituent gray and white tissue types using a constrained fuzzy algorithm based on voxel intensity and tissue boundaries; and (4) measurements of gray and white total brain tissue volumes (total brain tissue equals gray plus white tissue). These procedures have been described and validated elsewhere.<sup>30</sup>

### STATISTICAL ANALYSIS

Distributions were checked for normality and homogeneity of variances. Analyses of total brain tissue, total gray matter, and total white matter were performed using 1-way analyses of variance (ANOVAs), with diagnosis (VCFS vs controls) as a between-subject factor. Post hoc analyses using the Scheffé test were performed to compare further between VCFS subgroups of different parental origin (paternal vs maternal 22q11.2 microdeletion) and controls. Regression analyses were used to test for predictive relationships between age and gray matter volumes. A *P* value of .05 (2-tailed) was considered significant. Follow-up comparison of correlation coefficients was conducted using Fisher transformations<sup>31</sup> with a *P* value of .05 (1-tailed).

sis,<sup>16</sup> making VCFS the highest known risk factor identified to date for development of this psychiatric disorder, and supporting the notion of VCFS as a potential genetic-developmental model for schizophrenia.

Despite evidence of abnormal brain development in VCFS,<sup>22,23</sup> only one quantitative brain imaging study has been reported to date.<sup>24</sup> That study demonstrated volumetric reductions of total brain volume, left parietal lobe, and right cerebellum adjusted for total brain volume in 15 subjects with VCFS compared with 15 normal control subjects. Observation of tissue composition in this sample revealed a significant overall decrease of total gray and white matter tissue in subjects with VCFS. A qualitative investigation of brain morphology also described alterations among 11 subjects with 22q11.2 deletion and schizophrenia,<sup>25</sup> suggesting increased frequency of midline anomalies such as cavum vergae and brain atrophy.

**Table 1. Demographic Characteristics of Study Subjects**

	Subjects With VCFS*		Control Subjects (n = 18)
	22q11.2 Deletion on Maternal Allele (n = 9)	22q11.2 Deletion on Paternal Allele (n = 9)	
Age, mean $\pm$ SD, y	12.1 $\pm$ 2.9	11.8 $\pm$ 3.9	12.5 $\pm$ 3.8
Sex, male/female	6:3	5:4	11:7
Frequency of heart malformation, No. of subjects	7	6	0

\*VCFS indicates velocardiofacial syndrome.

To date, investigations have failed to reveal any effect of parental origin of the deletion on the physical phenotype or degree of cognitive impairment in affected individuals.<sup>26</sup> However, recent studies reported that familial transmission

**Table 2. Gray Matter and White Matter Volumes\***

	Subjects With VCFS		Control Subjects (n = 18)
	22q11.2 Deletion on Maternal Chromosome 22 (n = 9)	22q11.2 Deletion on Paternal Chromosome 22 (n = 9)	
Total gray matter, cm <sup>3</sup>	691.5 ± 48†	762.8 ± 67‡	785.5 ± 59§
Total white matter, cm <sup>3</sup>	406.9 ± 36	446.2 ± 58¶	523.7 ± 72#

\*Data are given as mean ± SD. VCFS indicates velocardiocardial syndrome.

†Post hoc Scheffé test vs controls,  $P = .005$ .

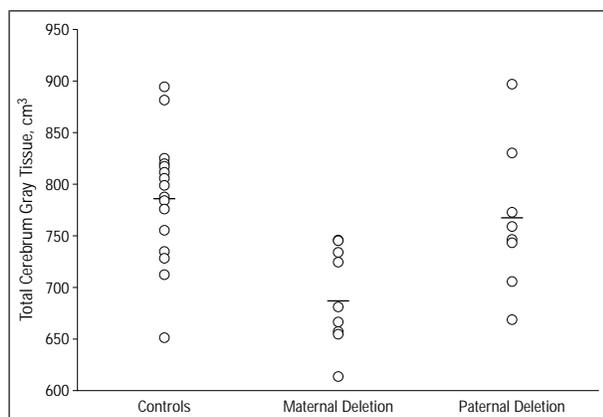
‡Post hoc Scheffé test vs controls,  $P = .64$ .

§Analysis of variance vs both VCFS subgroups,  $P = 0.02$ .

||Post hoc Scheffé test vs controls,  $P < .001$ .

¶Post hoc Scheffé test vs controls,  $P < .05$ .

#Analysis of variance vs both VCFS subgroups,  $P < .001$ .



**Figure 1.** Differences in total cerebrum gray tissue volume between subjects with maternal and paternal 22q11.2 deletion and normal control subjects.

of the disorder was associated with lower cognitive performance.<sup>26,27</sup> This finding suggests a genetic mechanism leading to a transgenerational deterioration, but genetic evidence for this has not been reported. On the other hand, investigators have found that parental origin of a genetic deletion can have a significant effect on the physical and cognitive phenotype of genetic disorders (eg, Angelman and Prader-Willi syndrome caused by a deletion of 15q11 or uniparental disomy of chromosome 15). This observation relates to a mechanism of gene expression regulation referred to as *imprinting*. Imprinting of a gene means that this gene is expressed in a manner that depends on the parent of origin of the chromosome, thus resulting, eg, only in expression of the gene located on the maternally derived chromosome.

We present the first evidence that the parental origin of the 22q11.2 deletion has a significant effect on brain development and morphology, and we discuss the potential impact of this finding on the association between schizophrenia and VCFS.

## RESULTS

Similar to results of a previous study from our laboratory,<sup>24</sup> total brain tissue volume was approximately 11% smaller (ANOVA;  $F_{1,34} = 22.0$ ;  $P < .001$ ) in the VCFS group

( $1154 \pm 97$  cm<sup>3</sup>) relative to controls ( $1309 \pm 102$  cm<sup>3</sup>). Gray ( $F_{1,34} = 7.6$ ;  $P < .01$ ) and white ( $F_{1,34} = 22.0$ ;  $P < .001$ ) matter contributed to this difference.

The ANOVA comparisons (**Table 2**) indicated a significant difference between the control and VCFS subgroups when comparing total volume of cerebral gray ( $F_{2,33} = 7.8$ ;  $P < .01$ ; **Figure 1**) and white ( $F_{2,33} = 12.2$ ;  $P < .001$ ) matter. When both subgroups were compared with the controls (follow-up Scheffé tests), the VCFS subgroup with maternal-origin deletions showed significantly decreased volumes of gray ( $P < .005$ ) and white ( $F_{2,33} = 12.2$ ;  $P < .001$ ) matter compartments. In contrast, the subgroup with deletions of paternal origin showed only decreased cerebral white matter relative to controls ( $P < .05$ ). Finally, the follow-up Scheffé tests comparing VCFS subgroups indicated that children with the deletion on the maternal chromosome 22 had significantly decreased ( $P < .05$ ) gray matter volume but no significant difference in white matter volume ( $P = .41$ ).

Regression analyses indicated that age significantly predicted gray matter volume decrease only for subjects with maternal-origin deletions ( $R^2 = 0.58$ ;  $P = .02$ ; **Figure 2**). Using a follow-up Fisher *r*-to-*z* transformation, the age–gray matter correlations of controls and subjects with VCFS with the deletion on the maternal chromosome 22 were compared; this comparison did not reach statistical significance ( $z = 1.61$ ;  $P = .11$ ).

## COMMENT

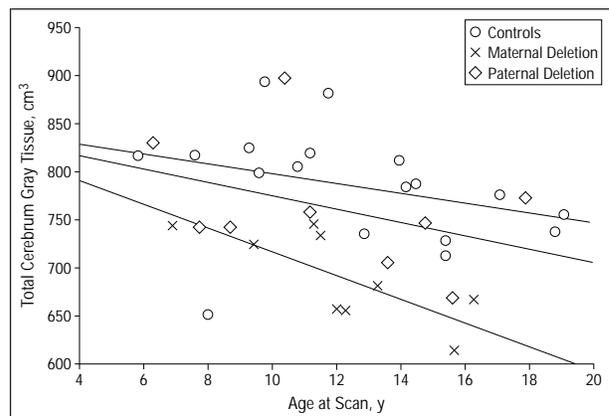
A previous publication<sup>24</sup> showed that children and adolescents with VCFS experience reduction of gray and white matter tissue volumes. To date, no study has investigated the potential impact of parental origin of the deletion on brain development in VCFS. Our results indicate a significant effect of the parental origin of 22q11.2 microdeletions on brain development in children and adolescents with VCFS (Figure 1). Subjects who inherit their unique set of 22q11.2 genes through the paternal germ line (ie, who have a deletion on the maternal chromosome 22) have reduced volumes of gray matter compared with normal control subjects and patients inheriting these genes only from the maternal germ line. Imprinting and inhibition of the expression of at least 1 gene affecting neuronal proliferation or cell death, dendritic arborization, or creation and elimination of synapses is a possible explanation for this phenomenon. Because white matter tissue tends to be reduced independent of parental origin of the deletion, it seems likely that haploinsufficiency of another distinct gene(s) also coded in the 22q11.2 region is responsible for this effect. Clear limitations of this preliminary study reside in the limited sample sizes and the cross-sectional nature of data.

Previous evidence that genetic paternal imprinting affects neuronal and brain development has been demonstrated in 2 lines of research. First, support for paternal genetic imprinting is derived from knowledge of another genetic disorder, Angelman syndrome. Persons with this condition, due to paternal uniparental disomy (UPD, abnormal inheritance of both homologue chromosomes from the same parent) for chromosome 15 or maternal deletion of 15q11.1-12, manifest severe mental retardation, ab-

sent speech, ataxia, jerky limb movement, and inappropriate laughter.<sup>32</sup> Neuropathology studies<sup>33,34</sup> of Angelman syndrome show decreased dendritic arborization and reduction in number of dendritic spines of pyramidal neurons in cortical layers 3 and 5. Second, animal models have provided additional information regarding the involvement of imprinting mechanisms in normal brain development. The creation of maternal and paternal disomic chimeras in mice has been made possible recently through experimental chromosomal rearrangement during early embryonic development.<sup>35-37</sup> These chimeras are embryos consisting of a mixture of cells maternally disomic/normal (ie, a mixture of maternally disomic cells and normal cells [mixed populations]) or paternally disomic/normal. Observations of cerebral and neuronal development in these chimeric mice have suggested that, compared with normal mice and paternal chimeras, augmented development of neurons in the neocortex and the striatum occurs in maternal chimeras. Further, compared with normal littermates, brains of mice with maternal disomy are enlarged, likely the result of double-expressed gene dosage, whereas brains with paternal disomy are reduced in volume. These findings in animal models are consistent with reduction of gray matter in subjects with VCFS who have deletion of the maternal allele as described herein, and suggest an essential role for maternally expressed alleles in cortical and telencephalic development.

The number of publications reporting UPD and the effect of parental imprinting on chromosome 22 is very limited,<sup>38</sup> with only a single case report of paternal UPD.<sup>39</sup> No obvious impact of UPD of chromosome 22 on clinical phenotype has been detected. However, these reports did not investigate brain development and morphology, and did not assess cognitive abilities using standardized tools.<sup>39-42</sup> Similarly, previous studies looking at the molecular pathophysiology or physical phenotype of VCFS have not pointed to an imprinting effect. Although studies have observed that familial transmission of the disorder results in more severe intellectual disabilities than de novo cases,<sup>26,27</sup> investigators have not explained this finding adequately. It is possible that this observation is a consequence of a recruitment bias of subjects with a maternally deleted deletion, because women with VCFS seem more likely to reproduce than men and are more likely to be identified when a parent of a child with VCFS is affected.<sup>16,26</sup>

The differential effect of parental origin of 22q11.2 deletion on gray matter development has several implications for research on VCFS as well as schizophrenia. First, discovery of a potentially imprinted gene contributing to decreased gray matter in VCFS considerably narrows the number of gene candidates whose hemizygosity is responsible for neuronal development in the 22q11.2 region. Second, smaller gray matter volume exhibited by children with maternal origin of the deletion may place these subjects at increased risk for childhood- or adult-onset schizophrenia. Recent publications in schizophrenia have demonstrated the potential significance of cortical gray matter reduction and support the hypothesis of a premorbid neurodevelopmental etiologic process. Adolescents with childhood-onset schizophrenia experienced a 4-fold decrease of cortical gray matter relative to their normal coun-



**Figure 2.** Developmental paths of gray matter volumes of subjects with maternal and paternal 22q11.2 deletion and normal control subjects. The uppermost regression line shows gray matter volume changes with age in normal controls; the middle line, in subjects with velocardiofacial syndrome (VCFS) due to a paternally derived deletion; and the lowermost line, in subjects with VCFS due to a maternally derived deletion.

terparts.<sup>43</sup> In patients with adult-onset schizophrenia, gray matter volume reduction is already evident at first clinical presentation of the disorder<sup>44,45</sup> and appears to explain at least partially the morbid<sup>44,46,47</sup> and premorbid cognitive features<sup>48</sup> associated with this condition. If VCFS, like schizophrenia, is a developmental disorder associated with excessive gray matter reduction, it could provide a potential model for studying etiologic pathways leading to schizophrenia or associated neuropsychiatric disorders. Parental origin of predisposing alleles should be considered when constructing genetic models for schizophrenia, particularly as pertaining to the potential influence of imprinting on the pathogenesis of this neuropsychiatric disorder<sup>49</sup> and on cortical development.<sup>50</sup>

The small subsample sizes of the present study represent an important limitation and necessitate replication with larger groups. In addition, because of statistical power limitation, we did not investigate subregions of the brain to further identify potential differences in neuroanatomical patterns among VCFS subgroups and controls. Future studies will need to validate our preliminary results using larger samples and to investigate potential differences in brain development using longitudinal data. Identification of the parental origin of 22q11.2 microdeletion in adult subjects with VCFS and schizophrenia awaits future research that integrates neuroanatomy, cognition, and psychopathology.

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