Brain structure and function in velo-cardio-facial syndrome with and without psychosis
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CHAPTER 5

BRAIN ANATOMY IN ADULTS WITH VELO-CARDIO-FACIAL SYNDROME WITH AND WITHOUT SCHIZOPHRENIA: PRELIMINARY RESULTS OF A STRUCTURAL MAGNETIC RESONANCE IMAGING STUDY

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ABSTRACT

Background: Velo-cardio-facial syndrome is associated with interstitial deletions of chromosome 22q11, mild / borderline learning disability, characteristic dysmorphism, and a high prevalence of schizophrenia. The biological basis to this increased risk for schizophrenia is unknown, but it has been suggested that people with velo-cardio-facial syndrome may have genetically determined differences in brain anatomy which predispose to the development of psychosis. However, to date there are no neuroimaging studies comparing schizophrenic and non-schizophrenic adults with velo-cardio-facial syndrome. Methods: We studied brain anatomy in 39 adults using quantitative structural magnetic resonance imaging: 13 with velo-cardio-facial syndrome and schizophrenia (mean age (± SD) 34 yrs ± 11, IQ 69 ± 8), 12 with velo-cardio-facial syndrome without history of a psychosis (mean age 31 yrs ± 10, IQ 74 ± 9), and 14 age-IQ matched healthy controls. Data were analysed using both manual tracing and computerised voxel-based methods. Results: People with velo-cardio-facial syndrome and schizophrenia, compared to both the controls and non-schizophrenic velo-cardio-facial syndrome people, had a significant (p<0.05) reduction in volume of whole brain brain matter (white + grey) and whole brain white matter; and an increase in total and sulcal cerebrospinal fluid volume. Regional grey and white matter differences were most pronounced in frontal regions of the schizophrenic people. Both velo-cardio-facial syndrome groups had a reduced cerebellar volume compared to controls. Conclusions: Within velo-cardio-facial syndrome, schizophrenia is associated with generalised differences in brain anatomy, but white matter and frontal regions may be particularly implicated. These abnormalities may reflect genetically determined abnormalities in neurodevelopment. Studies with larger samples are needed to replicate our findings.
INTRODUCTION

Velo-cardio-facial syndrome (VCFS) is a genetic disorder which occurs in approximately 1:2500 - 1:4000 live births. In 85% of people with VCFS a ~3Mb deletion of 22q11 is detected with fluorescence in-situ hybridisation (FISH)\(^2\). VCFS is associated with a characteristic physical phenotype (including congenital cardiovascular anomalies, and cleft lip / palate), mild / borderline learning disability, and specific cognitive deficits (e.g. in object perception, planning, and abstract reasoning) independent of their IQ\(^1,6\). In addition, psychiatric problems are frequently reported in VCFS children. These include social withdrawal, phobia, depression, attention deficit hyperactivity disorder and autistic spectrum disorder\(^7,8\). In adult life, people with VCFS are at increased risk of developing psychosis particularly schizophrenia\(^9\). The reported prevalence rates for psychosis vary between 10-64 % but methodological differences complicate the interpretation of these studies\(^9,12\). In a large study Murphy et al.\(^9\) found psychosis in 30% (schizophrenia in 24%) of adults with VCFS.

Schizophrenia is a heterogenous disorder which is likely to be caused by interaction of several susceptibility genes and environmental risk factors. The morbid risk of schizophrenia for a patient with VCFS is approximately 25 times the general population risk and thus possession of chromosome 22q11 deletion, apart from being the offspring of two schizophrenic parents or having a schizophrenic monozygotic co-twin, is the highest known risk factor for the development of schizophrenia. This suggests that deletion of one or more gene(s) mapping to chromosome 22q11, underlies susceptibility to psychosis in VCFS\(^13\). The study of VCFS therefore provides a unique opportunity to increase our understanding of the neurobiology of schizophrenia in the general population.

There is growing consensus from a large body of in vivo neuroimaging studies that people with schizophrenia have several structural brain abnormalities. Increased volume of cerebral ventricles is one of the most consistently reported findings, together with reduction in the volume of total brain and grey matter (for a review see 14). Also, localized volume and grey matter reductions have frequently been described in several brain regions – mostly implicating temporo-limbic and frontal neocortical regions\(^15,16\). In contrast, there have been fewer studies on white matter volumes, but diffusion tensor imaging (DTI)\(^17-19\), and magnetic transfer imaging (MTI) studies\(^20,21\) and micro-array data\(^22,23\) suggest that white matter integrity, including oligodendroglia and myelination may be compromised in schizophrenia\(^24\).

There are relatively few neuroimaging studies of people with VCFS. Qualitative studies have found that people with VCFS have a high prevalence of white matter hyperintensities (WMHIs, which may reflect abnormalities in myelination and high water content), septum pellucidum abnormalities, and a small cerebellar vermis\(^25-29\). Quantitative studies reported that learning disabled VCFS children (without psychosis) when compared with healthy normal intelligence children have a generalised reduction in volume of both cerebral hemispheres (mostly affecting white matter), combined with increased volume of frontal lobe and
decreased volume of left parietal grey matter\textsuperscript{10,31}. There are only two quantitative neuroimaging studies in adults with VCFS. We reported that, when compared to IQ matched controls, VCFS adults have a smaller cerebellar volume, widespread deficits in white matter, and localised grey matter deficits in temporal and cerebellar regions\textsuperscript{32}. Our study, however did not differentiate between VCFS individuals with or without schizophrenia. Recently, Chow et al.\textsuperscript{33} compared adults with VCFS and schizophrenia to healthy non-VCFS controls, and reported that VCFS adults with schizophrenia had; a smaller volume of total grey matter; regional differences in grey and white matter in frontal, temporal, parietal and occipital lobes; and an increased volume of ventricular and sulcal CSF bilaterally. Although this study was an important first step, their control group was not matched for the presence of VCFS or IQ, and so it is unclear if the differences they reported are due to the presence of VCFS, schizophrenia, learning disability – or all three.

To our knowledge, there have been no studies comparing the brain anatomy of VCFS adults with and without schizophrenia. We therefore extended our previous work and examined the brain structure of schizophrenic and non-schizophrenic adults with VCFS and chromosome 22q11 deletion, and a healthy IQ matched control group using structural magnetic resonance imaging (MRI). We tested the hypothesis that 1) VCFS adults with schizophrenia have a significant reduction of brain volume in frontal and temporo-limbic brain regions reflecting brain abnormalities associated with psychosis in the general population; 2) both VCFS adults with and without schizophrenia would have a significant reduction of cerebellar volume reflecting a brain abnormality specific for VCFS.

SUBJECTS AND METHODS

Subjects:
Approval for the study was granted by the local ethics committee, and all subjects gave written informed consent after the procedure was fully explained. All subjects were screened for medical conditions affecting brain function using a semi-structured clinical interview and routine blood tests. Also, a semi-structured psychiatric interview was performed (Schedules for Clinical Assessment in Neuropsychiatry)\textsuperscript{34} to establish a DSM-IV (American Psychiatric Association, 1994) diagnosis using a methodology described elsewhere\textsuperscript{6}. Full scale intelligence (FSIQ) was measured using the Canavan shortened version of the Wechsler Adult Intelligence Scale - Revised comprising of five subtests: Vocabulary, Comprehension, Similarities, Block Design, and Object Assembly\textsuperscript{15}. People with VCFS were recruited from the University of Wales College of Medicine, Cardiff and the Behavioural Genetics Clinic, Institute of Psychiatry, London. We included 25 subjects with clinical features of VCFS and a 22q11 deletion detected by fluorescence \textit{in situ} hybridisation (FISH) (Oncor Inc. Gaishersburg, MD 20877, USA). The VCFS group was subdivided into two: those who met DSM-IV criteria for schizophrenia (n=13, 7 females / 6 males, age 34 years (SD 11), IQ 69 (SD 8), all on antipsychotic medication and duration of illness > 1 year, 2 of which hospitalized at time of scanning) and those who had no history of psychosis (n=12, 8 females / 4 males, 31 years (SD 10) and IQ 74 (SD 9)). A third, healthy control group was included,
recruited from local community centres for people with mild or borderline learning disabilities (n = 14, 8 females / 6 males, 36 years (SD10) and IQ 75 (SD 16)).

**Image acquisition**

MRI of the brain was performed on a GE Signa 1.5 Tesla system (General Electric, Milwaukee, WI, USA) at the Maudsley Hospital London, UK. A coronal volumetric spoiled grass (SPGR) data set covering the whole head was acquired (repetition time (TR) = 13.8 ms, echotime (TE) = 2.8 ms, 124 slices, 1.5 mm slice thickness). This dataset was used to perform manual tracing of brain volumes\(^\text{36}\). In addition, we acquired a whole brain near axial dual - echo fast spin-echo (FSE) data set aligned with the anterior commissure (AC) - posterior commissure (PC) plane (TR = 4000 ms, effective TE = 20 and 85 ms, 3mm slice thickness). This dataset was used to determine between-group differences in grey and white matter volume using a previously published methodology \(^\text{32,37,38}\). Three types of analysis were performed, one qualitative and two quantitative; all blind to subject group status.

**Qualitative analysis**

Both MRI datasets were assessed qualitatively by a neuroradiologist. The presence and extent of ventricular WMHIs was assessed using a four point rating scale adopted from Kozachuk \(^\text{25}\) as follows: grade 0 = ventricular WMHIs absent; grade 1 = frontal or occipital caps or pencil thin lining of the lateral ventricle; grade 2 = smooth halo surrounding the lateral ventricles; and grade 3 = irregular ventricular WMHIs extending into the deep white matter. Deep WMHIs were graded as follows: grade 0 = absent; grade 1 = punctuate foci either focal or symmetrical; grade 2 = mild confluence of foci; and grade 3 = large confluence of foci. Peripheral WMHIs were graded similarly to deep WMHI. Congenital abnormalities in cerebellum and cerebrum were also noted as being present or absent.

**Manual tracing**

As described previously, volumetric analysis of total and regional brain areas was performed on a reformatted SPGR dataset using Measure software \(^\text{36}\). Total, right, and left caudate, putamen, hippocampus, amygdala, frontal, occipito-parietal, and temporal lobe, cerebral hemispheres, and cerebellum, brainstem, ventricular cerebrospinal fluid (CSF) volume were traced using region of interest boundaries as previously described \(^\text{32,37,39,41}\). The volume of each region was calculated by multiplying the summed pixel cross-sectional areas by slice thickness. Intra-rater and inter-rater reliabilities (range 0.90-0.99) were determined for all brain regions traced by the operators and were highly significant (F>4.0 and P<0.002) \(^\text{42}\).

**Voxel-wise analysis**

Voxels representing extracerebral tissue were automatically set to zero\(^\text{33}\) and the probability of each intracerebral voxel belonging to grey matter, white matter, CSF, or dura / vasculature tissue classes was then estimated by a modified fuzzy clustering algorithm\(^\text{44}\). On the basis of prior results, we equated these probabilities to the proportional volumes of each tissue class in the often heterogeneous volume
of tissue represented by each voxel. Thus, for example, if the probability of grey matter class membership was 0.8 for a given voxel, it was assumed that 80% of the tissue represented by that voxel was grey matter. Because the voxel size was predetermined (2.2 mm³), we then estimated the volume in millilitres of grey matter, white matter and CSF in each voxel. Summing these voxel tissue class volumes over all intracerebral voxels yielded global tissue class volumes.

To allow estimation of between-group differences at each intracerebral voxel, the short echo (proton-density-weighted) FSE images were co-registered using an affine transformation with a template image in the coordinate system of standard space as defined by Talairach & Tournoux. This individually estimated transformation was then applied to each of that subject’s grey and white tissue probability maps.

**Statistics:**

**Qualitative data:**

Group differences in frequencies of structural abnormalities were assessed using Fisher's Exact test, whereas between group differences in extent of WMHIs were assessed using a univariate general linear model (GLM), with level of significance for both tests at p < 0.05.

**Analysis of MRI data using manual tracing:**

The analysis of manually traced volumes (Measure) was carried out using SPSS (SPSS 10.0 for Windows, SPSS Inc., Chicago, Illinois, USA). Data were first examined for normality to conform to the assumptions of the parametric statistics employed. Between-group differences in uncorrected total regional brain volumes were calculated using a univariate GLM with group (schizophrenic VCFS (S-VCFS) or non-schizophrenic VCFS (NS-VCFS), controls) and gender (male, female) as the between-subject variables, and age, and total intracranial volume (ICV) as covariates and where appropriate Bonferroni adjustments for multiple comparisons. The significance level was defined as p < 0.05.

**Analysis of MRI data using computerized voxel-wise analysis:**

FSE data were unavailable for 2 of the S-VCFS and 2 of the control subjects. Total grey and white matter and CSF volumes in the S-VCFS and NS-VCFS, and control groups were compared by univariate GLM controlling for gender, age, and ICV (SPSS 10.0) and where appropriate Bonferroni adjustments for multiple comparisons. Between-group differences in grey and white matter were localized by fitting an appropriate GLM at each intracerebral voxel. Inference was via a permutation distribution of spatially informed statistics with significance levels set to control for multiple comparisons by having less than one estimated false positive regions (clusters) across the image (p<0.001). In brief the processing proceeded as follows. Maps of the standardised GLM model coefficient of interest (group) at each voxel were thresholded such that only voxels with probability < 0.05 were retained. The sum of voxel wise statistics for each three-dimensional suprathreshold cluster was the test statistic, the sign indicating a relative excess or deficit in local tissue density. Significance testing of the clusters was performed...
using a null distribution of this test statistic similarly obtained after repeatedly randomly permuting the relevant factor in the GLM and refitting of the model⁹.

RESULTS

Qualitative (radiological) findings

The prevalence of septum pellucidum abnormalities was not equally distributed over the 3 groups: 31% S-VCFS subjects, 50% in NS-VCFS subjects and 0% in the control group (Fisher’s Z = 8.2, p = 0.02). However, there were no significant between-group differences in any other qualitative variable we measured or in severity of WMHIs (Table 1).

Brain volumes analysed using manual tracing

There was a significant group effect (F (2, 32) = 3.4, p = 0.04) and gender (F (1, 32) = 10.8, p = 0.002) effect on ICV, but no group by gender interaction. Pairwise comparisons with Bonferroni adjustments for multiple comparisons showed a significant decreased ICV in the S-VCFS group compared to the control group and in the female compared to the male group. In addition after adding ICV as a covariate to the model, there was a significant group effect on volume of total (F (2, 31) = 6.08, p = 0.006), left (F (2, 31) = 7.99, p = 0.002) and right (F (2, 31) = 5.1, p = 0.01) cerebral hemispheres; total (F (2, 31) = 5.4, p = 0.01), left (F (2, 31) = 3.3, p = 0.05) and right frontal lobe (F (2, 31) = 5.5, p = 0.009); total (F (2, 31) = 5.1, p = 0.01), left (F (2, 31) = 6.02, p = 0.006), and right temporal lobe (F (2, 31) = 3.35, p = 0.05), cerebellum (F (2, 31) = 14.1, p = 0.0005), brainstem (F (2, 31) = 4.02, p = 0.03) and total sulcal CSF (F (2, 31) = 9.3, p = 0.001) (table 2). Pairwise comparisons with Bonferroni adjustments for multiple comparisons revealed that cerebellar volume was significantly smaller in both S-VCFS and NS-VCFS groups compared to controls. Furthermore, total and left cerebral hemisphere volume were significantly smaller and sulcal CSF volume significantly larger in the S-VCFS compared to both NS-VCFS and control groups. Decreases in volume of right hemisphere, total and right frontal lobe; total, left and right temporal lobe; and brainstem volume were observed in the S-VCFS group compared to the control group only. There was a significant effect of age on total (F (1, 31) = 9.14, p = 0.005), left (F (1, 31) = 6.36, p = 0.02) and right (F (1, 31) = 12.02, p = 0.002) frontal lobe, but no age x group interactions. There were no significant effects of age and gender on any of the other brain structures.
Table 1 Qualitative (radiological) findings in S-VCFS, NS-VCFS and healthy controls

<table>
<thead>
<tr>
<th>Structure</th>
<th>S-VCFS (n=13)</th>
<th>NS-VCFS (n=12)</th>
<th>Controls (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small / abnormal cerebellar vermis</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cavum septum pellucidum/vergæ</td>
<td>4</td>
<td>6</td>
<td>0*</td>
</tr>
<tr>
<td>Total presence of WMHI</td>
<td>8</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Ventricular WMHI</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Deep WMHI</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Peripheral WMHI</td>
<td>7</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Mean rating ventricular WMHI (SD)</td>
<td>0.89 (0.3)</td>
<td>0.5 (0.9)</td>
<td>0.08 (0.29)</td>
</tr>
<tr>
<td>Mean rating deep WMHI (SD)</td>
<td>0.9 (1.2)</td>
<td>1.0 (1.5)</td>
<td>0.33 (0.65)</td>
</tr>
<tr>
<td>Mean rating peripheral WMHI (SD)</td>
<td>1.5 (1.3)</td>
<td>0.6 (1.2)</td>
<td>0.42 (0.67)</td>
</tr>
</tbody>
</table>

WMHIs = white matter hyperintensities

* p<0.05 Fisher’s exact
<table>
<thead>
<tr>
<th>Brain Structure</th>
<th>S-VCFS (n=13)</th>
<th>NS-VCFS (n=12)</th>
<th>Controls (n=14)</th>
<th>P - value, df = 2, 31</th>
<th>Effect size</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total intracranial volume</td>
<td>1239.68 (181.22)</td>
<td>1298.40 (118.00)</td>
<td>1373.42 (124.24)</td>
<td>0.04*</td>
<td>0.18</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Hemispheres</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>928.13 (99.96)</td>
<td>1012.57 (89.06)</td>
<td>1065.62 (98.73)</td>
<td>0.006**</td>
<td>0.28</td>
<td><strong>0.85</strong></td>
</tr>
<tr>
<td>Left</td>
<td>458.23 (48.52)</td>
<td>500.02 (42.75)</td>
<td>524.83 (48.02)</td>
<td>0.002**</td>
<td>0.34</td>
<td><strong>0.94</strong></td>
</tr>
<tr>
<td>Right</td>
<td>463.73 (47.10)</td>
<td>507.22 (47.58)</td>
<td>534.79 (53.03)</td>
<td>0.01**</td>
<td>0.25</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Frontal lobes</strong></td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>463.06 (49.60)</td>
<td>501.31 (41.73)</td>
<td>528.82 (53.22)</td>
<td>0.01**</td>
<td>0.26</td>
<td><strong>0.81</strong></td>
</tr>
<tr>
<td>Left</td>
<td>227.24 (26.60)</td>
<td>245.94 (20.94)</td>
<td>255.75 (24.14)</td>
<td>0.05*</td>
<td>0.18</td>
<td>0.58</td>
</tr>
<tr>
<td>Right</td>
<td>233.66 (21.82)</td>
<td>253.87 (22.81)</td>
<td>269.48 (31.93)</td>
<td>0.009**</td>
<td>0.26</td>
<td><strong>0.82</strong></td>
</tr>
<tr>
<td><strong>Occipito-parietal lobes</strong></td>
<td>346.24 (49.02)</td>
<td>380.78 (51.73)</td>
<td>395.61 (44.2)</td>
<td>0.30</td>
<td>0.08</td>
<td>0.26</td>
</tr>
<tr>
<td>Left</td>
<td>175.16 (25.65)</td>
<td>190.40 (28.77)</td>
<td>201.53 (21.13)</td>
<td>0.40</td>
<td>0.06</td>
<td>0.2</td>
</tr>
<tr>
<td>Right</td>
<td>170.65 (24.68)</td>
<td>189.88 (24.15)</td>
<td>203.94 (30.72)</td>
<td>0.19</td>
<td>0.10</td>
<td>0.34</td>
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<tr>
<td><strong>Temporal lobes</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>118.61 (16.17)</td>
<td>132.28 (18.05)</td>
<td>144.0 (15.07)</td>
<td>0.01**</td>
<td>0.25</td>
<td>0.78</td>
</tr>
<tr>
<td>Left</td>
<td>57.75 (8.29)</td>
<td>65.02 (8.41)</td>
<td>71.09 (6.93)</td>
<td>0.006**</td>
<td>0.28</td>
<td><strong>0.85</strong></td>
</tr>
<tr>
<td>Right</td>
<td>60.86 (9.10)</td>
<td>67.26 (10.12)</td>
<td>72.92 (8.82)</td>
<td>0.05*</td>
<td>0.18</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Putamen</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.98 (1.56)</td>
<td>6.69 (0.79)</td>
<td>7.6 (3.13)</td>
<td>0.40</td>
<td>0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>Left</td>
<td>3.60 (0.92)</td>
<td>3.38 (0.37)</td>
<td>3.92 (0.66)</td>
<td>0.29</td>
<td>0.08</td>
<td>0.26</td>
</tr>
<tr>
<td>Right</td>
<td>3.39 (0.73)</td>
<td>3.31 (0.46)</td>
<td>3.71 (0.73)</td>
<td>0.64</td>
<td>0.03</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Caudate</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Total</td>
<td>7.69 (1.49)</td>
<td>8.05 (0.92)</td>
<td>8.08 (1.14)</td>
<td>0.82</td>
<td>0.01</td>
<td>0.08</td>
</tr>
<tr>
<td>Left</td>
<td>3.85 (0.76)</td>
<td>4.04 (0.44)</td>
<td>4.04 (0.48)</td>
<td>0.86</td>
<td>0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>Right</td>
<td>3.85 (0.75)</td>
<td>4.03 (0.51)</td>
<td>4.04 (0.73)</td>
<td>0.79</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Lateral ventricles</strong></td>
<td>26.57 (39.61)</td>
<td>14.08 (4.90)</td>
<td>20.76 (11.85)</td>
<td>0.07</td>
<td>0.16</td>
<td>0.52</td>
</tr>
<tr>
<td>Left</td>
<td>13.62 (21.04)</td>
<td>7.11 (2.53)</td>
<td>11.27 (6.23)</td>
<td>0.09</td>
<td>0.15</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Total and regional brain volumes (ml). Values are group means (SD); * p<0.05, **p<0.01

**Tissue class volumes analysed using computerized voxel-wise analysis**

Total tissue class volumes are shown in table 3. There was no significant effect of group on total grey matter volume. However, there was a significant effect of group on volume of total white matter (F(2, 26) = 8.73, p = 0.001) and total CSF (F(2, 26) = 8.57, p = 0.001). Pairwise comparisons with Bonferroni adjustments for multiple comparisons revealed that total white matter volume was significantly decreased and total CSF volume significantly increased in the S-VCFS group compared to both the NS-VCFS and the control group. Also, there was a significant effect on age for total grey matter (F (1, 26) = 12.12, p = 0.002) and white matter (F (1, 26) = 10.56, p = 0.003), and an gender x group interaction for white matter (F (2, 26) = 3.88, p = 0.03) (with males in the S-VCFS group showing a greater reduction in white matter volume than females).

**Spatial extent statistics.** The central coordinates and volumes of the three-dimensional clusters of brain tissues that were significantly different (p=0.001) are shown in table 4. The S-VCFS group compared to the control group had six significant grey matter deficit regions, two in (left and right) cerebellum, one in right superior temporal gyrus, and three in right frontal regions (mid-frontal, inferior frontal, anterior cingulate gyrus). Also, one grey matter excess region was identified in the S-VCFS group; this was centered in the right anterior cingulate gyrus (figure 1).
### Table 3 Tissue class volumes in S-VCFS, NS-VCFS and controls.

<table>
<thead>
<tr>
<th>Tissue class (ml)</th>
<th>S-VCFS (n=11)</th>
<th>NS-VCFS (n=12)</th>
<th>Controls (n=11)</th>
<th>P value, d.f. 2, 26</th>
<th>Effect size</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey matter</td>
<td>584.83 (91.48)</td>
<td>597.38 (87.14)</td>
<td>590.12 (61.47)</td>
<td>0.94</td>
<td>0.005</td>
<td>0.06</td>
</tr>
<tr>
<td>White matter</td>
<td>534.82 (70.42)</td>
<td>569.01 (79.70)</td>
<td>643.82 (98.16)</td>
<td><strong>0.001</strong></td>
<td>0.40</td>
<td>0.95</td>
</tr>
<tr>
<td>CSF</td>
<td>213.88 (86.30)</td>
<td>158.62 (38.73)</td>
<td>162.03 (38.58)</td>
<td><strong>0.001</strong></td>
<td>0.40</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Total tissue class volumes (ml). Values are group means (SD); **p<0.01

---

**Figure 1**

Relative deficits (blue) and excesses (red) in grey matter volume in S-VCFS people compared with healthy IQ matched controls. The maps are oriented with the right side of the brain shown on the left side of each panel. The z-coordinate for each row of axial slices in the standard space of Talairach & Tournoux (1988) is given in millimetres.

The NS-VCFS compared to the control group had one grey matter deficit region that was located in right cerebellum extending to left cerebellum (figure 2). In addition there were three grey matter excess regions all centered in the precentral regions, both left and right. Within-VCFS group comparisons revealed two grey matter excess regions in the S-VCFS compared to NS-VCFS group located in left precentral regions. There were no grey matter deficit regions in the S-VCFS group compared to the NS-VCFS group.
Figure 2
Relative deficits (blue) and excesses (red) in grey matter volume in NS-VCFS people compared with healthy IQ matched controls. The maps are oriented with the right side of the brain shown on the left side of each panel. The z-coordinate for each row of axial slices in the standard space of Talairach & Tournoux (1988) is given in millimetres.

White matter deficits in the S-VCFS group compared to the control group were concentrated in four spatially extensive regions all covering frontal lobe regions: two involving left and right right precentral gyrus, one extending to left anterior cingulate, and one involving right medial frontal region (figure 3). In contrast, one area of excess white matter volume was observed centered in the brainstem of the S-VCFS group.
Figure 3
Relative deficits (blue) and excesses (red) in white matter volume in S-VCFS people compared with healthy IQ matched controls. The maps are oriented with the right side of the brain shown on the left side of each panel. The z-coordinate for each row of axial slices in the standard space of Talairach & Tournoux (1988) is given in millimetres.

In the NS-VCFS group, compared to controls, one cluster of white matter deficit was identified and this was centered in right fasciculus longitudinalis superior extending into right inferior frontal lobe (figure 4). Also, in the NS-VCFS group, significant excess white matter was localised in two clusters, -left and right fasciculus occipitofrontalis. Within VCFS group comparisons revealed one cluster of white matter excess in the S-VCFS group compared to the NS-VCFS group located in the posterior cingulate; there were no white matter deficit regions.

Figure 4
Relative deficits (blue) and excesses (red) in white matter volume in NS-VCFS people compared with healthy IQ matched controls. The maps are oriented with the right side of the brain shown on the left side of each panel. The z-coordinate for each row of axial slices in the standard space of Talairach & Tournoux (1988) is given in millimetres.
Table 4 Coordinates of regional differences in grey and white matter volume in VCFS adults with and without schizophrenia compared to IQ-matched controls

<table>
<thead>
<tr>
<th>Cerebral region</th>
<th>Brodmann area</th>
<th>n</th>
<th>Tal (x)</th>
<th>Tal (y)</th>
<th>Tal (z)</th>
<th>Side</th>
</tr>
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<tbody>
<tr>
<td><strong>S-VCFS - controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Grey Matter Deficit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1814</td>
<td>542</td>
<td>-10.3</td>
<td>49.0</td>
<td>14.4</td>
<td>L</td>
</tr>
<tr>
<td>Frontal: anterior cingulate</td>
<td>32</td>
<td>1133</td>
<td>3.8</td>
<td>42.7</td>
<td>6.6</td>
<td>R</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>47</td>
<td>329</td>
<td>34.0</td>
<td>13.0</td>
<td>-18.2</td>
<td>R</td>
</tr>
<tr>
<td>mid frontal gyrus</td>
<td>10</td>
<td>8</td>
<td>38.2</td>
<td>48.5</td>
<td>4.2</td>
<td>R</td>
</tr>
<tr>
<td><strong>Temporal: superior temporal gyrus</strong></td>
<td>22</td>
<td>15</td>
<td>45.6</td>
<td>0.7</td>
<td>-3.8</td>
<td>R</td>
</tr>
<tr>
<td><strong>Grey Matter Excess</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal: medial frontal gyrus</td>
<td>24</td>
<td>4774</td>
<td>1.2</td>
<td>-7.7</td>
<td>45.9</td>
<td>R,L</td>
</tr>
<tr>
<td><strong>White Matter Deficit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal: precentral regions</td>
<td>575</td>
<td>392</td>
<td>-34.9</td>
<td>-5.4</td>
<td>42.6</td>
<td>R</td>
</tr>
<tr>
<td>Superior/mid frontal regions</td>
<td></td>
<td>245</td>
<td>13.8</td>
<td>53.5</td>
<td>3.4</td>
<td>R</td>
</tr>
<tr>
<td>Medial frontal</td>
<td>273</td>
<td>-24.9</td>
<td>42.8</td>
<td>-3.5</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td><strong>White Matter Excess</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain stem</td>
<td>4079</td>
<td>0.9</td>
<td>-36.1</td>
<td>-11.4</td>
<td>R,L</td>
<td></td>
</tr>
<tr>
<td><strong>NS-VCFS - controls</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td><strong>Grey Matter Deficit</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1360</td>
<td>-4.0</td>
<td>-61.0</td>
<td>-12.1</td>
<td>L</td>
<td></td>
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<tr>
<td><strong>Grey Matter Excess</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal: precentral gyrus</td>
<td>4</td>
<td>228</td>
<td>39.1</td>
<td>-5.4</td>
<td>47.5</td>
<td>R</td>
</tr>
<tr>
<td>precentral gyrus</td>
<td>4</td>
<td>228</td>
<td>-42.3</td>
<td>-8.3</td>
<td>43.5</td>
<td>L</td>
</tr>
<tr>
<td>precentral gyrus</td>
<td>4</td>
<td>11</td>
<td>32.2</td>
<td>-20.3</td>
<td>59.5</td>
<td>R</td>
</tr>
<tr>
<td><strong>White Matter Deficit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasciculus longitudinalis superior</td>
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<td>33.7</td>
<td>15.5</td>
<td>13.5</td>
<td>R</td>
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<tr>
<td><strong>White Matter Excess</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasciculus occipito-frontalis</td>
<td>1067</td>
<td>-24.0</td>
<td>39.3</td>
<td>17.0</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>Fasciculus occipito-frontalis</td>
<td>830</td>
<td>24.3</td>
<td>-41.3</td>
<td>21.9</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td><strong>S-VCFS - NS-VCFS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Grey Matter Excess</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal: precentral gyrus</td>
<td>4</td>
<td>378</td>
<td>-21.3</td>
<td>-24.2</td>
<td>58.8</td>
<td>L</td>
</tr>
<tr>
<td>medial frontal gyrus</td>
<td>6</td>
<td>17</td>
<td>-4.7</td>
<td>-23.4</td>
<td>58.2</td>
<td>L</td>
</tr>
<tr>
<td><strong>White Matter Excess</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>posterior cingulate</td>
<td>173</td>
<td>0.7</td>
<td>-38.3</td>
<td>24.8</td>
<td>R,L</td>
<td></td>
</tr>
</tbody>
</table>

Location of each cluster’s centroid is given in Talarich coordinates (Tal x, y, z, mm), n = number of voxels in each cluster. The clusterwise probability is p = 0.001
DISCUSSION
We believe that this is the first (preliminary) study to investigate brain anatomy in VCFS individuals with and without schizophrenia using an IQ matched healthy control group as a reference. We found that both S-VCFS and NS-VCFS groups have a smaller volume of cerebellum and increased frequency of septum pellucidum abnormalities. In addition, the S-VCFS group (compared to both control and NS-VCFS groups) had a significant reduction in volume of both total (white + grey) brain matter and white matter in whole brain. The S-VCFS group also had a significant increase in volume of total and sulcal CSF. It is unlikely that our findings can be explained by differences in IQ, age, gender or non-specific factors associated with being learning disabled, as the groups did not differ in IQ and we controlled for age and gender distribution.

Qualitative analysis of our data revealed abnormalities that have been previously reported in people with VCFS and in non-VCFS related schizophrenia. Cavum septum pellucidum was observed in both VCFS groups equally frequently irrespective of whether schizophrenia was present. Septum pellucidum abnormalities are of unknown clinical significance and are also seen in people with and without learning disabilities without mental illness (although they were not present in our control group). The presence of WMHIs in the majority of subjects in both VCFS groups at a relatively young age could indicate white matter tract disruption possibly of a vascular origin, although we did not use a method that is sensitive to fiber tract visualization, like DTI. However, in our study these abnormalities were equally common in VCFS people with and without schizophrenia; and there were no significant between-group differences in frequency or severity of WMHIs. In addition, WMHIs were also present in the control group. Thus, WMHIs and septum pellucidum abnormalities are frequently present in VCFS individuals with and without schizophrenia and are most likely not related to the presence / absence of a psychotic illness.

Our quantitative analysis revealed that the S-VCFS group, compared to both the NS-VCFS and control groups have a generalized reduction in total brain (grey + white matter) volume and total white matter volume and an increase in total and sulcal volume of CSF. However, we also found that the S-VCFS group (as compared to the control group only) had a reduction in volume of the frontal, and temporal lobes. These findings are in agreement with results from other MRI studies of schizophrenia in both the general population and in children and adults with VCFS.

In contrast to a prior report in people with VCFS, we did not find a significant diffuse loss of grey matter in adults with S-VCFS. We suggest that our results differ from those of Chow et al. because they included non-VCFS controls with an above average level of intellectual functioning (mean IQ 116). Thus the differences they observed in total grey matter volume could have been confounded by differences in subject intelligence and health status – e.g. IQ is positively correlated with cortical grey matter volume.
Both S-VCFS and NS-VCFS groups had a smaller total cerebellar volume and cerebellar grey matter compared to the control group. This finding further supports the results from our previous work and other qualitative and quantitative studies in VCFS children. Others have suggested that in the general population psychosis is associated with cerebellar abnormalities. Our results suggest that cerebellar abnormalities are specific to people with VCFS, however these are not related to the presence / absence of psychosis.

In contrast, the S-VCFS group demonstrated generalized loss of total brain (grey + white) and total white matter volume ‘together with an increase in total and sulcal CSF volume’ as compared to both NS-VCFS and control groups. In addition, our quantitative analysis revealed regional differences in grey and white matter. These were most pronounced in frontal lobe regions in S-VCFS adults. Prior MRI studies in children and adults with VCFS reported differences in development of frontal lobe. Moreover, abnormalities in the function, structure and metabolism of frontal regions have been frequently reported in the non-VCFS schizophrenic population. For example, both grey and white matter deficits have been reported in frontal brain regions and findings from longitudinal MRI studies suggest a progressive volume reduction in frontal regions, including frontal white matter. Normal brain maturation takes place last in the frontal regions during adolescence and is accompanied by both increased synaptic pruning and myelination - leading to a reduction of volume in grey matter and increase in white matter. We found that people with S-VCFS had a reduction in whole brain volume, but with regional differences in grey and white matter particularly affecting frontal regions. Thus our findings might suggest that people with VCFS may have abnormalities in frontal maturation, and this could predispose to the development of schizophrenia. We therefore suggest that 22q11 deletion is associated with the presence of a generalised disturbance in brain development which increases the liability for developing psychosis. However psychosis may only develop when regionally specific neurodevelopmental differences of frontal regions also occur - leading to changes in the volume and tissue composition of frontal regions.

We did not find support for our hypothesis that people with S-VCFS have a reduced volume of hippocampus, but found reduced temporal grey matter and temporal lobe volume compared to the healthy control group. This finding is in agreement with, results by Chow et al. who found reduced temporal grey matter volumes in their sample of adults with VCFS and schizophrenia, compared to normal IQ controls, a finding also frequently reported in the non-VCFS associated schizophrenia.

The cause of the differences in brain anatomy of people with VCFS, its relation to schizophrenia and the time course of their development, is unknown. Our study was cross-sectional and therefore we cannot determine if these differences in brain anatomy change across the lifespan. Nonetheless we found that sulcal CSF volume was increased in S-VCFS adults and this adds tentative support to the hypothesis that schizophrenia is associated with an abnormal neurodevelopmental process that
is not limited to an aberration in prenatal brain development, but progresses even after postnatal brain volume expansion is complete. Also, our results do not allow us to draw conclusions as to whether schizophrenia in VCFS is due to a primary abnormality in grey matter development (e.g. differences in programmed cell death (apoptosis)), with secondary changes of white matter structure, or vice versa. There is preliminary evidence to suggest that differences in programmed cell death may underlie the differences in brain anatomy we found in our study. For example, recent studies reporting that PRODH2, a gene located at 22q11, is implicated in apoptosis and that variation within the PRODH2/DGR6 locus at 22q11 might contribute to VCFS associated schizophrenia, though the latter study has not been replicated.

Alternatively, schizophrenia in VCFS may be related to developmental differences in white matter structure (e.g. possibly as a result of abnormal myelination) with consequent disturbances in connectivity between neocortical and limbic grey matter regions. Schizophrenia in the general population is increasingly seen by some as a supraregional disorder involving differences in interconnecting white matter tracts and neuronal connectivity possibly associated with oligodendroglial dysfunction resulting in abnormalities in myelin maintenance and repair. In favour of this argument is the finding that in children with VCFS (who are at risk of developing psychosis) white matter may be more compromised than grey matter, and WMHs are common. Also, we found overall white matter to be compromised in S-VCFS whereas there were no between-group differences in total grey matter volume.

Another potential candidate gene for schizophrenia in VCFS is the catechol-O-methyltransferase gene (COMT; the enzyme that degrades dopamine). COMT lies within the 22q11 region and therefore people with VCFS have a reduced gene dosage of COMT. Also, a recent study demonstrated that variation in activity of COMT, may have neurobiological effects specific to the prefrontal cortex and modulate the risk for schizophrenia in the general population. Moreover, others have suggested that in schizophrenia neurodevelopmental abnormalities of prefrontal dopaminergic systems result in enhanced vulnerability to sensitization during late adolescence and early adulthood. Although there is little evidence yet that COMT plays a major role in the development of schizophrenia in VCFS, it cannot be excluded that reduced gene dosage of COMT affects the integrity of the dopaminergic system, including its prefrontal projections and so increases susceptibility for schizophrenia in people with VCFS.

Our sample size and the cross-sectional design is a limitation of this study, but we were still able to detect several significant differences with a large effect size and sufficient power, and as noted by others the effect size for structural brain abnormalities in this group of patients is relatively large. In addition we carried out multiple statistical comparisons and thereby increased the risk of a type I error (false positive outcomes). However, we feel this is unlikely to fully explain our results. Where possible, adjustments for multiple comparisons were made. Also, we found significant effects on manually traced brain volumes (e.g. reduced cerebellar volume) that were consistent with our computerized voxel-wise analysis. Moreover, in our
computerized voxel-wise analysis of grey and white matter volumes, the level of significance adopted was chosen specifically to yield less than one false-positive cluster. We did not control for effects of neuroleptic medication, parental IQ and parental head circumference and therefore we cannot exclude these as potential confounds of our results. Also, we did not quantify white matter lesions and did not relate them to other outcome variables.

In conclusion, our results, although preliminary, suggest that structural brain abnormalities present in people with S-VCFS are partially similar to those seen in people with schizophrenia in the general population. Our findings are compatible with an abnormal neurodevelopmental process which continues even after postnatal brain volume expansion has been completed. We suggest that this results in generalised abnormalities in brain anatomy, but particularly affecting white matter and frontal brain regions. Larger and longitudinal studies are planned to replicate these findings, and to determine how genetic and environmental variables are related to the development of schizophrenia in VCFS.
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