Neuropathologic Features in Adults with 22q11.2 Deletion Syndrome

The 22q11.2 deletion syndrome (22qDS) is the most common microdeletion syndrome in humans. Its multisystem manifestations include congenital anomalies and neuropsychiatric disorders such as schizophrenia. Structural neuroimaging shows various abnormalities, but no postmortem brain studies exist. We report neuropathologic findings in 3 individuals from a cohort of 100 adults with a confirmed 22q11.2 deletion. All 3 had schizophrenia. Postmortem examination of Case 1, a 44-year-old male, revealed bilateral periventricular nodular heterotopia in the frontal lobes and ectopic neurons scattered throughout the frontal white matter. Cases 2 (male, aged 22 years) and 3 (female, 52 years) showed no evidence of migration abnormalities, but both had extensive astrocytic gliosis and focal collections of macrophages in the cerebral white matter, suggestive of cerebrovascular pathology. Review of magnetic resonance imaging findings available for 66 other subjects in the cohort revealed polymicrogyria in one and right cerebellar disorganization in another of the 26 subjects with schizophrenia. The results support previous neuroimaging reports suggesting that neuronal migration abnormalities may be a feature of 22qDS. Both early developmental brain abnormalities and fetal and later microvascular pathology may play a role in the pathogenesis of the neuropsychiatric phenotype of 22qDS, including white matter abnormalities and schizophrenia.

Keywords: neuronal migration, pathology, schizophrenia, velocardiofacial syndrome

Introduction

The 22q11.2 deletion syndrome (22qDS), also known as velocardiofacial syndrome or DiGeorge syndrome, is the most common human microdeletion syndrome, occurring in approximately 1 in every 4000 live births (Oskarsdottir et al. 2004). The phenotype is highly variable, with congenital and later onset features, classically including mild dysmorphic features, velopharyngeal insufficiency, and congenital cardiac defects. There is a wide range of cognitive impairments, including generalized learning difficulties and specific deficits (Chow et al. 2006). 22qDS is also associated with behavioral and psychiatric abnormalities, including a markedly elevated risk for schizophrenia (Bassett and Chow 1999), which affects about 25% of adults with 22qDS (Bassett et al. 2005). The typical clinical presentation suggests that 22qDS is a syndromal subtype of schizophrenia (Bassett et al. 2003), accounting for about 1% of schizophrenia in the general population (Horowitz et al. 2005). Patients with 22qDS most commonly have an ~3 Mb hemizygous 22q11.2 deletion, indicating that factors other than deletion length likely play a role in the variable clinical expression of the syndrome, including that of schizophrenia (Weksberg et al. 2007).

Neuroimaging studies have shown variable and often subtle changes in brain anatomy in 22qDS. Common findings include reduction of overall brain volume (van Amelsvoort et al. 2004), midline defects such as cavitum septum pellucidum (Chow et al. 1999), alterations in the size of the corpus callosum (Shashi et al. 2004; Simon et al. 2005), and T2-bright foci (white matter hyperintensities) on magnetic resonance imaging (MRI) (Chow et al. 1999; van Amelsvoort et al. 2004; Campbell et al. 2006). Reductions in cerebellar volume have also been described (Chow et al. 1999; Bish et al. 2006; Campbell et al. 2006). As in general populations of schizophrenia (Harrison 1999), there is some evidence that increased ventricular and sulcal cerebrospinal fluid and decreased temporal gray matter volumes are associated with schizophrenia in 22qDS (Chow et al. 2002; van Amelsvoort et al. 2004). Major brain malformations are rare in 22qDS. Polymicrogyria with a perisylvian and right-sided predilection has been found in 32 cases with moderate to severe mental retardation and/or significant neurological signs (Robin et al. 2006). Abnormal patterns of cortical gyration (Bird and Scambler 2000; Schaeer et al. 2005) and rarely neural tube defects (Nickel and Magens 1996) have also been reported.

To date, there are no reports of neuropathologic postmortem examinations of patients with molecularly confirmed 22q11.2 deletions and 22qDS. We report the postmortem neuropathologic findings in 3 patients from a cohort of 100 adults with 22qDS and place the results in the context of available neuroimaging data for the cohort. We discuss the possible implications for our understanding of abnormal brain development and neuropsychiatric manifestations in this common but underrecognized syndrome.

Materials and Methods

Sample, Clinical Assessments, and Brain Imaging

We follow a cohort of 100 adults (>17 years) diagnosed with 22qDS and confirmed to have a chromosome 22q11.2 deletion by standard methods using fluorescence in situ hybridization (FISH) techniques and a probe, most commonly TUPLE1 (Vysis) or N25 (ONCOR), from the 22q11.2 region (Driscoll et al. 1993). As previously described (Scutt et al. 2001; Bassett et al. 2003, 2005), we have comprehensive medical and psychiatric phenotypic data on this cohort. Sixty-eight subjects (28 males, 40 females; mean age 26.6 years, standard deviation 10.0 years) had brain MRI using a GE Signa 1.5-T scanner (GE Healthcare, Milwaukee, WI) and previously described methods (Chow et al. 1999, 2002). Images were systematically reviewed for qualitative anomalies by a research neuroradiologist (DM) blind to the clinical status of the subjects (Chow et al. 1999). Informed consent to participate in this longitudinal study was obtained in writing, and the study approved by the Research Ethics Boards of the University of Toronto, Centre for Addiction and Mental Health, and University Health Network.
Pathology

Three subjects with 22qDS from the cohort had postmortem brain tissue available for study: Cases 1 (male, 44 years), 2 (male, 22 years), and 3 (female, 52 years). Complete postmortem autopsies were performed after postmortem intervals of 26, 6, and 12 h, respectively. Gross examination of the brains from Cases 1 and 2 was performed after 2 weeks of fixation in 10% buffered formalin. The cerebral hemispheres were sectioned coronally and the cerebellum and brain stem sectioned perpendicular to the axis. Case 3 had a forensic autopsy with dissection of the brain in the fresh state, formalin fixation, and subsequent gross examination of multiple fragments of brain from different regions (see Results).

For light microscopy, standard-sized samples were taken from 25 different brain regions. The 5-μm-thick sections were stained with Luxol fast blue–hematoxylin and eosin (LH&E) and cresyl violet (CV) stains. On selected 5-μm-thick sections, immunohistochemistry for CD68 (Dako PGM1 clone, concentration 1:50; Dako, Carpinteria, CA), synaptophylin (prediluted polyclonal antibody; Cell Marque, Rocklin, CA), NeuN (Chemicon, concentration 1:400; Chemicon, Temecula, CA), and glial fibrillary acidic protein (GFAP; predilute from Ventana, Tuscon, AZ) was performed using an avidin–biotin complex, peroxidase–anti-peroxidase staining. Selective 5-μm-thick sections were stained with the antibodies for synaptophysin (prediluted polyclonal antibody; Cell Marque, Rocklin, CA), CD68 (Dako PGM1 clone, concentration 1:50; Dako, Carpinteria, CA), and glial fibrillary acidic protein (GFAP; predilute from Ventana, Tuscon, AZ) was performed using an avidin-biotin complex, peroxidase-based method. All sections were counterstained with hematoxylin. For all slides, controls included known positive material and negative controls by omission of the primary antibody. The extent of gliosis was assessed by immunohistochemistry for GFAP on sections from the same region in the deep frontal white matter and was compared with 3 age-matched normal controls that served as reference cases. To quantify findings, image analysis of GFAP immunoreactivity was assessed in Adobe Photoshop Elements 5.0 using the histogram tool. The average luminosity of images in Figure 9 was measured, and the staining intensity was expressed as [1000]/[luminosity value]. An average of images in Figure 9 was measured, and the staining intensity was expressed as [1000]/[luminosity value]. An average normal intensity was derived from the 3 reference cases, and the 3 cases were compared with this average, expressed as a percentage.

Results

The neuropsychiatric phenotype and neuropathologic findings of the 3 cases are summarized in Table 1 and portrayed in Figures 1-3 (Case 1), 4-6 (Case 3). All had the common ~3 Mb 22q11.2 deletion.

Case Descriptions and General Autopsy Findings

Case 1

This 44-year-old Caucasian man of English-Canadian descent had onset of psychosis at age 18 years and was first hospitalized with symptoms meeting DSM-IV criteria for schizophrenia at age 21. The illness had a deteriorating course with multiple hospitalizations, poor compliance to treatment, and cognitive and functional decline. Intellect was documented to be in the low average range at age 21 and in the low borderline range at age 34. He was chronically hospitalized from the age of 35 with grossly disorganized thinking, affect and behavior, unpredictable violent outbursts, poor self-care, as well as grandiose delusions and auditory hallucinations resistant to standard antipsychotic treatments and electroconvulsive therapy. An electroencephalogram (EEG) at age 42 indicated generalized cortical and subcortical dysfunction but no epileptiform activity, consistent with a diffuse encephalopathy. Soon after this, water intoxication, with hyponatremia and hypochloremia, was noted and fluid restriction recommended. He had a single generalized tonic-clonic seizure 6 months later during a 2-week episode of ataxia and delirium secondary to complex metabolic disturbances (hypoxia, hypercapnia, hypothyroidism, and hypocalcemia). Computed tomography scan revealed no intracranial abnormalities. Transfer to a specialized psychiatric hospital at age 43 and treatment with clozapine led to some...
improvement in mental and physical state. There was no history of drug- or alcohol-use disorders.

His pediatric history included an atrial septal defect surgically repaired at age 4 years, severe thoracolumbar scoliosis treated with rod insertion at age 11, and asthma. He had required special tutoring from age 11, achieving grade 12 at vocational school and 1 year of college. Adult medical history included asthma and chronic obstructive pulmonary disease, treated with multiple inhalers. Although he had quit smoking at age 40, poor treatment compliance and ongoing exposure to smoke contributed to periodic exacerbations requiring oxygen. Chronic sinus tachycardia was considered secondary to the respiratory condition and its treatment. He had been diagnosed with hypertension at age 37, hypothyroidism at age 39, and obesity at age 40.

When the patient was 42 years old, a psychiatrist noted typical facial features of 22qDS and hypernasality, leading to genetic assessment and a confirmed diagnosis of 22qDS on standard FISH testing using the TUPLE1 probe. The investigations that followed revealed hypocalcemia and relative hypoparathyroidism; treatment with calcium carbonate and vitamin D supplements was instituted. Echocardiogram showed concentric left ventricular (LV) hypertrophy, trivial mitral and tricuspid regurgitation, and normal LV function. Abdominal ultrasound showed splenomegaly.

The general autopsy revealed mild cardiovascular abnormalities, for example, biventricular hypertrophy and mild aortic atherosclerosis, but no obvious cause of death.

**Case 2**

This 22-year-old man of Filipino descent had a history of DSM-IV undifferentiated schizophrenia with onset of social withdrawal, auditory and visual hallucinations, inappropriate affect, and disorganized behavior at age 15 years. These symptoms were partially responsive to standard antipsychotic medication as an outpatient. At age 18, full remission of the psychotic illness and associated temper outbursts, secondary major depression, and school refusal was achieved on a combination of antipsychotic and antidepressant medications. For over 4 years until his death, he enjoyed school, leisure activities, and family life, coping well with multiple handicaps and health issues. These included mild mental retardation, compulsive skin picking, eczema, hypoparathyroidism, obesity, and gout. There was no history of epilepsy, head injury, or substance use. He had a single generalized hypocalcemic seizure at age 11 months. EEG at age 2 was within normal limits. Brain MRI at age 17 showed a single hyperintensity signal in the right hemispheric white matter (Fig. 4).

Neonatal hypocalcemia, congenital cardiac disease, developmental delays, and mild facial features led to a clinical diagnosis of DiGeorge syndrome in infancy. 22q11.2 deletion was confirmed at age 12 years on FISH testing using the D22S75 probe. His complex congenital cardiac disease included pulmonary atresia, nonrestrictive ventricular septal defect, right aortic arch and descending aorta, and absent central pulmonary arteries with multiple aortopulmonary collaterals providing pulmonary blood flow. He had significant symptomatic and functional improvement from the last of several palliative surgeries to dilate and stent these collaterals 58 months before death. Symptoms and functional status were stable at cardiac exam 5 months before death. However, echocardiogram revealed increased aortic valve stenosis, moderate aortic regurgitation, mildly decreased LV systolic function, moderately enlarged right ventricular cavity, and reduced global systolic function.

The general autopsy revealed severe congestive heart failure with a markedly enlarged heart and severe biventricular hypertrophy. Multiple congenital vascular anomalies were found, including atresia of the pulmonary valve and trunk, a large ventricular septal defect with an overriding aorta, and anomalous blood vessels between the proximal descending aorta and the lungs. The aortic valve was markedly calcified, which likely resulted in significant aortic stenosis. Various organs, including the brain, showed vascular congestion. An acute peripleural pulmonary infarct was found in the inferoposterior tip of the right lower lobe. The cause of death was multifactorial. Severe congestive heart failure, progressive valvular stenosis of the single ventricular outflow tract, pulmonary hypertension with alveolar hemorrhages, and severe abnormalities of the large and small pulmonary arteries clearly compromised cardiopulmonary functions. In this setting, relatively insignificant insults such as a small pulmonary embolus and/or a focal bronchopneumonia could have initiated cardiopulmonary failure.

**Case 3**

This 52-year-old woman of French-Canadian descent had a history of DSM-IV undifferentiated schizophrenia with onset of psychotic symptoms at about age 18 years. She had multiple hospitalizations for auditory hallucinations, delusions, and disorganized behavior. Compliance with prescribed treatments was often poor, symptoms responded only partially, and she had significant functional impairment. With antipsychotic and anxiolytic medications, irritability and temper outbursts were reduced, and she enjoyed some activities at her group home despite chronic auditory hallucinations. Chronic compulsive tearing of her fingernails led to periodic nail bed infections. There was no history of drug- or alcohol-use disorders, head injury, or seizures. An EEG at age 21 years showed no evidence of epileptiform activity. Brain MRI at age 46 showed multiple white matter hyperintensities in both hemispheres.

**Figure 1.** Computed tomography scan for Case 1 performed 2 years before death. It demonstrates focal calcifications in the left internal globus pallidus but otherwise normal findings. The resolution was not sufficient to detect the heterotopic nodules.
predominantly in subcortical regions (Fig. 7). Mild mental retardation and developmental delays were noted in early childhood, and the patient had received special education through school.

Pediatric history included a ventricular septal defect that closed spontaneously in early childhood, slight kyphosis with fused lower thoracic vertebrae, and asthma. Adult medical history included obesity, chronic smoking, and complications of asthma, such as episodes of hypoxia, hypercapnia, pneumonia, and respiratory failure requiring intubation 2 years before death. There was no history of hypertension.

During a psychiatric admission at age 46 years, a psychiatrist noted subtle facial features suggesting a genetic syndrome and referred the patient for a psychiatric genetics assessment, leading to a clinical diagnosis of 22qDS that was confirmed by FISH testing using the TUPLE1 probe. Subsequent investigations revealed a normal echocardiogram, asymptomatic gallstones on abdominal ultrasound, and hypocalcemia with relative hypoparathyroidism that was treated with calcium and vitamin D supplements.

A general postmortem examination revealed mild centrilobular emphysema and scattered areas of acute bronchopneumonia in both lungs. There was a small secundum-type atrial septal defect. There was no evidence of atherosclerosis in major coronary or cerebral arteries. Death was attributed to acute bronchopneumonia.

**Neuropathologic Findings**

**Case 1**
The prefixation brain weight was 1300 g. Externally, there was no evidence of abnormal gyration, such as polymicrogyria or pachygyria (Fig. 2A). Some relatively prominent gyri were seen in the posterior frontal lobe and anterior parietal lobe (Fig. 2A,B).

![Figure 2](http://cercor.oxfordjournals.org/)

**Figure 2.** Macroscopic neuropathology for Case 1. (A) The gyration pattern was normal, with no evidence of polymicrogyria or pachygyria. (B) Some relatively large gyri were present in the posterior frontal and anterior parietal lobes. (C) The base of the brain was unremarkable. (D) Coronal sections revealed BPNH along the lateral edge of both lateral ventricles (arrows), only seen in the frontal lobes. (E) White matter structures were unremarkable.
Figure 3. Histopathologic findings for Case 1. (A, B) Sections from the heterotopic nodules revealed disorganized gray matter (arrows). The frame corresponds to Figure 3C. (B) Smaller nodules were only visible microscopically (arrows). The ventricular surface was frequently denuded, and there was subependymal fibrosis. (C) Higher power view of the heterotopic nodule from Figure 3A reveals haphazard arrangement of neurons with a cortical-type morphology. (D) Large numbers of neurons (arrows) were found in the white matter between the heterotopic nodules and closest cortical surface. Magnification (insets) reveals cytologic details, such as triangular shape. (E) Immunohistochemistry for synaptophysin showed abundant reactivity in this white matter region. (F) The cortex over this area (right side) was poorly demarcated from the white matter, seen on the left side, which is only minimally stained with Luxol fast blue. (G) In the hippocampus, most of the neurons in the pyramidal layer showed a highly abnormal morphology. (H) Patches of ectopic gray matter resembling dentate nucleus were seen deep in the cerebellar white matter, magnified in the inset. (I) Scattered large ectopic neurons (arrows) were present individually in the white matter, magnified in the inset, or (J) at the interface (arrow) between the white matter and granular cell layer of the cerebellum (inset).
Abundant arachnoid granulations were present. The base of the brain was unremarkable (Fig. 2C). Coronal sections revealed bilateral periventricular nodular heterotopias (BPNHs) in the deep frontal white matter along the lateral edge of the lateral ventricles (Fig. 2D). In total, 7-8 of the nodules were visible grossly on each side, extending over 4 cm and corresponding to approximately 10 cm of overlying frontal cortex. There were also multiple spherical noncontiguous nodules that were only seen microscopically in the frontal lobes. Slightly more posterior sections demonstrated an unremarkable cortical ribbon and a normal-sized corpus callosum. There was no evidence of a cavum of the septum pellucidum (CSP) or cavum vergae. The cerebellar vermis and hemispheres had a normal size.

Microscopically, sections from the heterotopic nodules revealed disorganized gray matter (Fig. 3A; LH&E, ×5 magnification). Near the anterior tip of the left frontal horn, there were multiple smaller nodules (Fig. 3B, ×5). This section also demonstrated the extensive ependymal denudation and subependymal fibrosis seen along most of the ventricular surface. A higher power view of the nodule from Figure 3A shows that the neurons were haphazardly arranged but had a distinctly mature cortical morphology, such as pyramidal shape (Fig. 3C; LH&E, ×10). Large numbers of individual cortical-type neurons were also found in the white matter of the frontal lobe between the heterotopic nodules and the nearest cortical surface (Fig. 3D, arrows; ×10). In these areas, the white matter had a pale appearance on LH&E stains (Fig. 3D, ×20). This attenuation of myelinated fibers by neuropil-like substance was also confirmed with immunohistochemistry for synaptophysin (Fig. 3E, ×10). The cortex overlying this area was poorly demarcated from the white matter but appeared cytoarchitecturally unremarkable (Fig. 3F, ×5).

In the hippocampus, most of the neurons in the pyramidal layer, especially areas CA3 and CA4, showed a highly abnormal morphology (Fig. 3G, ×10). They had a round rather than triangular shape as well as a disorganized cytoarchitecture with very densely spaced neurons. Minor cytoarchitectural abnormalities were also detected in the cerebellum. Deep in the cerebellar white matter and distant from the deep nuclei, there were small isolated patches of ectopic gray matter with very densely spaced neurons. Minor cytoarchitectural abnormalities were also detected in the cerebellum. Deep in the cerebellar white matter and distant from the deep nuclei, there were small isolated patches of ectopic gray matter that resembled dentate nucleus (Fig. 3H, ×10). Neurons of similar size were also seen individually in the white matter (Fig. 3I; CV stain, ×10) or at the interface between white matter and granular cell layer (Fig. 3J, arrow and inset; CV, ×10). Other microscopic findings in this autopsy were leptomeningeal thickening, atherosclerosis, and vascular calcification in the left globus pallidus (compare to Fig. 1). No neuropathologic cause of death was found. Immunohistochemistry for GFAP performed on sections from the deep frontal white matter (Fig. 9A) showed a staining intensity that was similar to controls (Fig. 9D-F).

**Case 2**

The prefixation brain weight was 1305 g. The gyration pattern was normal throughout (Fig. 5A–C). The cerebral vasculature was very congested. Coronal sections revealed an anatomicocytoarchitecturally unremarkable brain (Fig. 5D,E). In a small segment at the level of the anterior thalamus, the corpus callosum was markedly thinned (Fig. 5E). There were no grossly visible lesions in the nearby white matter that could be linked to this finding. Other white matter structures, including the spot in the right frontal white matter where a $T_2$ MRI hyperintensity was visible at age 17 (Fig. 4, corresponding axial section), were also grossly unremarkable (Fig. 5E, arrow). A small posterior cavum vergae was noted (also see Fig. 4). There was no gross evidence of heterotopia or other migration defects. The cerebellum was macroscopically normal (not shown).

Microscopic sections from Case 2 revealed a largely unremarkable brain. There was no evidence of subtle migration defects, such as heterotopia, or disturbances in the cerebral cortico-cytoarchitecture. No ectopic neurons were seen in the deep frontal white matter or elsewhere. The area in the right frontal white matter corresponding to the hyperintensity on $T_2$ MRI (Fig. 4) was thoroughly sampled for histology. Slides revealed a focal area of pallor (Fig. 6A; ×5 LH&E), which on higher magnification showed abundant foamy (lipid-laden) macrophages (Fig. 6B, ×40), confirmed with an immunostain for CD68 (Fig. 6C). The cytoplasm has a slight blue color on the luxol stain, demonstrating the presence of myelin in macrophages. Many hemosiderin-laden macrophages were also present in this region, suggestive of microvascular abnormalities (Fig. 6A, arrow). The GFAP immunostain showed severe astrocytic gliosis in the deep frontal white matter (Fig. 9B). Staining intensity was increased by 33% over the average of 3 controls (Fig. 9F). A microscopic view of the segment with the very thin corpus callosum (Fig. 6D) shows white matter bundles with an unremarkable consistency. Specifically, there were no white matter lesions nearby. The hippocampus was cytoarchitecturally normal (Fig. 6F). Deep cerebellar nuclei, white matter, and cortex were also unremarkable. Other histologic findings in this case were leptomeningeal fibrosis and extensive ependymal denudation (Fig. 6I, arrow). The patient’s demise was explained by the findings in the general rather than the neuropathologic autopsy.

**Case 3**

The prefixation brain weight was 1080 g. Dissection was performed in the fresh state, and all tissues were subsequently
fixed in 10% buffered formalin. Available for neuropathologic examination were multiple pieces of brain tissue. The largest of these measured 12 × 8.5 × 1.5 cm and consisted of a slice of left frontal cortex and white matter as well as anterior basal ganglia and internal capsule (Fig. 8A). Multiple smaller slices from all lobes, brain stem, and cerebellum were also included. No macroscopic abnormalities were evident.

Microscopic sections from various regions demonstrated severe cerebrovascular hypertensive-type changes, for example, vascular thickening, perivascular pallor, abundant perivascular hemosiderin-laden macrophages (Fig. 8B), and multi-lumen formation in the gray matter. There was also some ependymal stripping at the surface of the lateral ventricles. There was no evidence of subtle migration defects, such as heterotopias, ectopic neurons, or disturbances in the cerebral cortical cytoarchitecture. No significant abnormalities were seen in sections from the pons or medulla oblongata. Immunohistochemistry for GFAP revealed severe astrocytic gliosis in the deep frontal white matter (Fig. 9C). There was an increase in staining intensity by 48.1% compared with controls (Fig. 9E).

MRI Findings
A review of MRI data available from the total cohort (n = 68) revealed 2 other subjects, both with schizophrenia, who also showed evidence of abnormal neuronal organization (see Table 1). Together with Case 1, whose findings would have been visible on MRI, this would represent 4.3% (3/69) of the sample with imaging data or equivalent available and 10% (3/30) of the subset with schizophrenia.

Discussion
This study represents the first report of postmortem neuropathologic findings in 22qDS, a syndrome with prominent central nervous system expression (Bassett et al. 2005). As expected, neuropathology revealed several new findings in 22qDS. In the 3 adults studied, where the most significant clinical neuropsychiatric feature was schizophrenia, neuropathologic examination revealed significant neuronal migration and/or vascular-related abnormalities. The results of this study provide initial pathological data contributing to our understanding of neuropsychiatric and neuroimaging features in 22qDS, particularly schizophrenia and white matter changes.

Abnormalities of Neuronal Migration, Organization, and Connectivity
Case 1 provided the most striking findings, including the first confirmed case of BPNH, a major malformation of cortical...
development, in 22qDS. Other microscopic neuronal migration abnormalities observed in the frontal lobe white matter, hippocampus, and cerebellum indicate widespread abnormalities of neurodevelopment in this patient with treatment-resistant schizophrenia. Although detailed neuropathologic examination of the other 2 cases studied did not find any evidence of neuronal disorganization, neuroimaging data for 2 of 66 other subjects from our adult 22qDS cohort, both with schizophrenia, showed disorganized cerebellar anatomy or polymicrogyria. MRI studies of 22qDS have previously shown polymicrogyria in some patients (Robin et al. 2006), including the only previous case with neuropathologic data based on a right temporal lobe neurosurgical specimen (Sztriha et al. 2004). All but 1 of the 32 reported cases with polymicrogyria were children, most with moderate to severe mental retardation, paresis, and/or seizures (Robin et al. 2006); outcome with respect to schizophrenia is unknown. Interestingly, these cases include 1 child also reported to have bilateral subcortical heterotopia on MRI (Koolen et al. 2004). Neuronal migration abnormalities may be associated with several different genetic abnormalities (Guerrini and Marini 2006). BPNH is in many cases caused by mutations in the filamin A gene at Xq28 and is found in several genetic syndromes (Guerrini and Marini 2006), including Williams syndrome with an extended 7q deletion (Ferland et al. 2006), and we would propose now 22qDS.

The results support the possibility that developmental neuronal disorganization and disturbances in neuronal connectivity may be involved in the pathogenesis of neuropsychiatric expression in 22qDS, including schizophrenia. In BPNH, clusters of gray matter are ectopically located along both lateral ventricles, reflecting a failure of cells to migrate out from the embryonic ventricular zone to the cortex or a failure of the later processes of apoptosis and dynamic cortical organization (Guerrini and Marini 2006). The neuronal migration abnormalities found in the current study were also bilateral, in contrast to the right-sided excess observed in polymicrogyria in 22qDS (Robin et al. 2006). The rate of BPNH in 22qDS schizophrenia (1/30) in the current study is comparable to that (1/55) found in a consecutively scanned sample of schizophrenia from the general population (Nopoulos et al. 1998), although it is unknown if the case with BPNH in the latter study had 22qDS. Comprehensive analyses of the hippocampus in some studies of schizophrenia (Highley et al. 2003) have not shown cytoarchitectural changes. However, other studies have found

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**Figure 6.** Histopathologic findings for Case 2. (A) Focal pallor and attenuation of myelinated fibers in the right frontal lobe. (B) Higher magnification showed abundant lipid-laden macrophages, many of which contain a blue tinge of myelin. (C) An immunostain for CD68 confirms the presence of macrophages. (D) The area of thin corpus callosum was microscopically unremarkable, except for focal ependymal stripping (arrow). (E) The hippocampus showed no cytoarchitectural abnormalities.
evidence of cortical disturbances of neuronal migration, organization, or morphology in schizophrenia (Harrison 1999; Benes and Berretta 2000), including in the hippocampus (Harrison 2004), some of which are similar to those seen in Case 1.

Case 1 also showed comparable developmental pathology in the cerebellum in the form of large heterotopic neurons (Fig. 3I,J). These could either represent dispersed neurons of the deep cerebellar nuclei or, alternatively, ectopic Purkinje cells that failed to attain a proper size. Similar pathology could underlie the disorganization of the right cerebellar white matter found in 1 subject with 22qDS from the MRI cohort. These neuropathologic findings may be consistent with abnormal cortical gyrification (Schaer et al. 2005) and a previously suggested vulnerability of the frontostriatal and cerebellar cortical networks in 22qDS (Kates et al. 2004; van Amelsvoort et al. 2004; Campbell et al. 2006). Unlike polymicrogyria (Robin et al. 2006), there is no indication that early vascular abnormalities play a role in the pathogenesis of BPNH or hippocampal or cerebellar neuronal disorganization. The majority of patients with schizophrenia, as for Cases 2 and 3, do not demonstrate histologic or imaging evidence of major neuronal migration abnormalities (Nopoulos et al. 1998; Harrison 1999). This does not rule out a role for less striking abnormalities of neuronal positioning and/or connectivity in 22qDS and/or schizophrenia. In schizophrenia, abnormal neural circuits are postulated to involve the glutamate neurotransmitter system and synapses, most likely as a result of abnormal neurodevelopment that may also include altered cerebral asymmetry (Harrison 2004). Similar to clinical neuropsychiatric expression (Chow et al. 2002, 2006; Bassett et al. 2003, 2005), specific pathological findings in 22qDS may be as variable as they are in general population samples of schizophrenia (Harrison 1999).

Abnormalities of neuronal migration are nonspecific and may be found with a broad range of neuropsychiatric features and severity (Guerrini and Marini 2006). It is possible that the developmental abnormalities observed may be unrelated to schizophrenia. BPNH is often, though not always, associated with seizure disorders; intellect may or may not be significantly affected (Guerrini and Marini 2006). However, Case 1 had only borderline learning difficulties and no history of epilepsy or other neurological disorder. The case of polymicrogyria identified from MRI data may be more associated with neurological and cognitive impairments than with schizophrenia. Adult psychiatric outcomes, however, are largely unknown in polymicrogyria (Guerrini and Marini 2006; Robin et al. 2006).

White Matter and Microvascular-Related Abnormalities

Microvascular-related and other white matter changes were prominent in Cases 2 and 3. White matter abnormalities are a common finding in neuroradiologic studies of 22qDS, including various abnormalities of the corpus callosum (Shashi et al. 2004; Simon et al. 2005; Machado et al. 2007), and $T_2$ MRI hyperintensities and white matter volume deficits in children (Campbell et al. 2006) and adults (Chow et al. 2002; van Amelsvoort et al. 2004). The significance of these findings is not understood, in part due to the lack of neuropathologic correlation. Case 2 had an MRI scan at age 17 that showed a prominent $T_2$-hyperintensity focus in the right frontal white matter (Fig. 4). Microscopically, this area contained numerous...
lipid-laden macrophages (Fig. 6A) and showed hypertensive-type vascular changes and perivascular hemosiderin-laden macrophages. These findings are suggestive of focal tissue destruction, in other words, a post-developmental process. A similar mechanism may also explain the focal thinning of the corpus callosum in this patient (Figs 5E and 6C). Therefore, caution appears warranted when interpreting subtle white matter abnormalities in 22qDS patients, such as volume loss, as in some cases these may be secondary to prior cerebrovascular events.

Immunohistochemistry for GFAP demonstrated severe astrocytic gliosis in Cases 2 and 3 and normal findings in Case 1 in the deep white matter. Generally, gliosis is not considered a feature of schizophrenia but rather a sign of coincidental pathological changes (Harrison 1999). However, gliosis may be present in periventricular regions and/or associated with the nonspecific focal degenerative changes (small infarcts and white matter changes) found in 50% of brains from patients with schizophrenia (Bruton et al. 1990). In the current study, gliosis is most likely to be related to the microvascular pathology and related tissue destruction that may be common in the general pathogenesis of 22qDS. Macroscopic cerebral artery anomalies observable in a minority (Chow et al. 1999) may be part of a broader spectrum of developmental microvascular anomalies in many patients with 22qDS. Vulnerability to later vascular-related events in the syndrome may be increased secondary to dysregulation of cerebral angiogenesis during development (Stalmans et al. 2003; Robin et al. 2006). Similar vulnerability due to neurodevelopmental changes has been proposed for schizophrenia (Bruton et al. 1990).

**Genes and Molecular Pathogenesis of 22qDS**

How may the findings of this study, which primarily implicate neuronal migration and cerebrovascular-related changes, relate to what is known about the molecular pathogenesis of 22qDS? The common ~3 Mb microdeletion on chromosome 22q11.2 occurs in about 90% of 22qDS patients (Saitta et al. 2004) and encompasses approximately 40 genes (Weksberg et al. 2007). Although mouse models of 22q11.2 deletions indicate that the brain is not grossly malformed (Lindsay et al. 1999), the altered gene dosage on chromosome 22 can affect expression in the developing and adult brain, for genes both within and outside the 22q11.2 deletion region (Meechan et al. 2006; Sivagnanasundaram et al. 2007). Many of the genes in the deletion region
are expressed during forebrain development in the mouse, several of which are candidate genes for schizophrenia (Maynard et al. 2003). Tbx1 and Crkl, for example, have important individual effects on behavior (Guris et al. 2001; Long et al. 2006; Paylor et al. 2006; Zweier et al. 2007) and interact with a regulator of telencephalic patterning, fibroblast growth factor 8 (Vitelli et al. 2002; Aggarwal et al. 2006; Moon et al. 2006). Dose-dependent interaction of Tbx1 and Crkl may affect local retinoic acid signaling (Guris et al. 2006), a central mechanism regulating neuronal migration, also proposed to be involved in the pathogenesis of schizophrenia (LaMantia 1999; Rioux and Arnold 2005). Tbx1 plays a key role in vascular development (Lindsay et al. 1999; Vitelli et al. 2002) and could be involved in microvascular-related neuropathology.

**Conclusion**

The neuropathologic examination of these adults with 22qDS revealed a number of abnormalities. Some of these are new observations, for example, BPNH and individual heterotopic neurons. Others confirm and provide new knowledge about previous neuroimaging data, for example, T2 MRI hyperintensities. The main limitation of the study is that the findings are based on 3 cases. While preliminary, it is tempting to conclude that the developmentally based neuropathologic findings may reflect the severity of the schizophrenic illness. Further human postmortem studies, in addition to animal studies, should allow for a better understanding of the dysregulated neuronal and microvascular organization, the genes involved, and their relation to normal brain development and the pathogenesis of schizophrenia in this important genetic syndrome.

**Note added in proof**

After the manuscript was accepted, a fourth patient (56-year-old female) from our 22qDS cohort with schizophrenia had a post-mortem examination. The results of the neuropathologic examination were consistent with the gliotic and microvascular pathology seen in cases 2 and 3, but not with the migrational pathology of case 1.

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**References**


Peyroux L, Arnold SE. 2005. The expression of retinoic acid receptor alpha is increased in the granule cells of the dentate gyrus in schizophrenia. Psychiatry Res. 133:13–21.


