Genetic Association and Brain Morphology Studies and the Chromosome 8p22 Pericentriolar Material 1 (PCM1) Gene in Susceptibility to Schizophrenia

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Context: There is evidence of linkage to a schizophrenia susceptibility locus on chromosome 8p21-22 found by several family linkage studies.

Objectives: To fine map and identify a susceptibility gene for schizophrenia on chromosome 8p22 and to investigate the effect of this genetic susceptibility on an endophenotype of abnormal brain structure using magnetic resonance imaging.

Design: Fine mapping and identification of a chromosome 8p22 susceptibility gene was carried out by finding linkage disequilibrium between genetic markers and schizophrenia in multiply affected families, a case-control sample, and a trio sample. Variation in brain morphology associated with pericentriolar material 1 (PCM1) alleles was examined using voxel-based morphometry and statistical parametric mapping with magnetic resonance imaging.

Setting and Patients: A family sample of 13 large families multiply affected with schizophrenia, 2 schizophrenia case-control samples from the United Kingdom and Scotland, and a sample of schizophrenic trios from the United States containing parents and 1 affected child with schizophrenia.

Main Outcome Measures: Tests of transmission disequilibrium between PCM1 locus polymorphisms and schizophrenia using a family sample and tests of allelic association in case-control and trio samples. Voxel-based morphometry using statistical parametric mapping.

Results: The family and trio samples both showed significant transmission disequilibrium between marker D85261 in the PCM1 gene locus and schizophrenia. The case-control sample from the United Kingdom also found significant allelic association between PCM1 gene markers and schizophrenia. Voxel-based morphometry of cases who had inherited a PCM1 genetic susceptibility showed a significant relative reduction in the volume of orbitofrontal cortex gray matter in comparison with patients with non–PCM1–associated schizophrenia, who, by contrast, showed gray matter volume reduction in the temporal pole, hippocampus, and inferior temporal cortex.

Conclusions: The PCM1 gene is implicated in susceptibility to schizophrenia and is associated with orbitofrontal gray matter volumetric deficits.

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The evidence of a major role for genetic susceptibility in the etiology of schizophrenia has been shown by family, twin, adoption, and genetic linkage studies. Rather than the family environment, specific, unique environmental factors, which include biological environment and stochastic effects that are not shared by siblings, have been implicated. Two types of investigation have dominated research into schizophrenia. The first type, using structural brain imaging studies, has consistently shown volumetric abnormalities in the brains of patients with schizophrenia. The other involves the systematic mapping of chromosomal regions in which genes that increase susceptibility to schizophrenia are present. A key finding has been the presence of locus heterogeneity with different genetic susceptibilities being involved in the etiology of schizophrenia in different multiply affected families.

Genetic linkage analyses to a chromosome 8p21-22 schizophrenia susceptibility locus (SCZD6, Online Mendelian Inheritance in Man [OMIM] 603013), under
the assumption of locus heterogeneity or admixture, confirm that this region of chromosome 8 harbors a susceptibility gene for schizophrenia. Three independent investigators report lod scores above 3.00 and a fourth confirms a lod score above 2.00. Once linkage has generally implicated a region of a chromosome, it is necessary to fine map exactly which gene is involved by tests of transmission disequilibrium and allelic association with schizophrenia to detect underlying linkage disequilibrium between marker alleles and disease alleles.

In an earlier attempt to fine map a specific susceptibility gene on 8p22, we carried out tests of allelic association in a smaller case-control sample with 15 markers that flanked the PCMI gene without finding any evidence of association. We then carried out tests of transmission disequilibrium of markers genotyped in our family study and followed up these results in 2 case-control samples and then in a collection of trios consisting of both parents and 1 affected offspring. Once allelic association due to linkage disequilibrium between markers and schizophrenia is detected, it is possible to differentiate genetic subgroups of schizophrenia and to examine clinical or endophenotypic characteristics of genotypically defined groups. We chose the endophenotype of abnormal brain morphology to study in relation to genotype because it seemed a priori the most likely variable to be associated with underlying heterogeneous genetic effects.

The evidence that there are volumetric deficits in the brains of subjects with schizophrenia has been replicated many times both in first-onset illness and in chronically affected subjects. Consistency is generally reported in the regional pattern of cortical and subcortical morphometric abnormalities affecting gray matter density and volume. The diagnosis of schizophrenia encompasses symptom clusters putatively associated with dysfunction of distinct neuroanatomical substrates. Methodological advances now enable unbiased automated analysis of structural magnetic resonance imaging (MRI) brain scans on a voxel-wide basis to quantify differences in brain morphometry and tissue composition at a level of detail that cannot be achieved by macroscopic examination. In this study, we describe an application of voxel-based morphometry (VBM) to test for differences in regional brain volume in patients with 8p22 PCMI–associated schizophrenia. A chromosome 8 subgroup was selected on the basis of having inherited specific marker alleles at the PCMI gene locus and compared with patients with schizophrenia without these alleles and with healthy matched control subjects.

METHODS

PATIENT AND CONTROL SAMPLES

The clinical sampling, diagnosis, and genotyping of the family sample for the testing of transmission disequilibrium between alleles at the D8S261 locus and schizophrenia has been described previously. Briefly, it consisted of 13 large, affected pedigrees containing cases diagnosed using Research Diagnostic Criteria (RDC). Pedigrees were selected on the basis of appearing to demonstrate a single source of schizophrenia or DSM-III-R schizotypal illness with unilineal transmission and no cases of bipolar affective disorder. A pedigree was considered unilineal not just on the basis of the interview diagnoses with the Schedule for Affective Disorders and Schizophrenia–Lifetime Version (SADS-L) but also with all the information available from past records. Two affection classes were used for the extended transmission disequilibrium test (ETDT) and linkage analyses: core schizophrenia consists of schizophrenia, unspecified functional psychosis, and schizoaffective psychosis; schizophrenia spectrum consists additionally of schizoid and schizotypal personality disorder according to DSM-III-R criteria. Of the 182 interviewed individuals, 56 fell into the core schizophrenia category and an additional 12 fell into the spectrum category.

The sampling of the UK case-control sample has recently been described in a study showing a genetic effect on schizophrenia from the region of the epsin 4 gene on chromosome 5. The UK sample consisted of 100 unrelated patients with schizophrenia and 200 unrelated “supernormal” controls from London and South England. This type of control, in which mental illness in the control subjects and their first-degree relatives is grounds for exclusion, will maximize the chance of finding a genetic difference between a schizophrenic group and a control group. All subjects in the case-control samples and the subsamples used for MRI scanning were included only if both parents were of white English, Irish, Welsh, or Scottish descent and if 3 out of 4 grandparents were of the same descent. One grandparent was allowed to be of Caucasian European origin but not of Jewish or non–European Union ancestry based on the European Union countries before the recent enlargement. Approval was obtained from the UK National Health Service multicenter and local research ethics boards, and all subjects signed an approved consent form after reading an information sheet.

All 450 schizophrenic cases were first selected for having a clinical diagnosis of schizophrenia made by a psychiatrist recorded in National Health Service medical case notes according to the International Classification of Diseases, 10th Revision (ICD-10), which is the same as the DSM-IV diagnosis of schizophrenia. Subjects with clear organic or symptomatic schizopreniform psychoses were excluded. All subjects were interviewed and data were collected with the SADS-L interview schedule. Accuracy of data entries into the SADS-L was assured by using medical case note information and all other available records. All cases were diagnosed as having schizophrenia according to the probable level of the RDC. The supernormal control subjects were also interviewed with the initial clinical screening questions of the SADS-L and selected on the basis of not having a family history of schizophrenia, alcoholism, or bipolar disorder and for having no past or present personal history of any RDC-defined mental disorder.

The US trio sample consisted of 100 unrelated patients meeting DSM-III-R criteria for schizophrenia or schizoaffective disorder as determined by structured diagnostic interview (Structured Clinical Interview for DSM-III-R [SCID]) conducted by trained raters with demonstrated interrater reliability. The research subjects were recruited from the Experimental Therapeutics branch of the National Institute of Mental Health and the Maryland Psychiatric Research Center of the University of Maryland School of Medicine, Baltimore. All subjects gave written informed consent to participate in the study under a protocol approved by the institutional review boards of the National Institute of Mental Health and the University of Maryland School of Medicine. After we obtained informed consent, the parents of each subject were also recruited for genetic testing.

The Scottish sample consisted of 200 unrelated cases of schizophrenia and 200 unrelated controls. Ethical approval was granted by the University of Edinburgh and the Royal Edinburgh Hospital, and all subjects gave informed written consent. These cases were diagnosed using the same clinical as-
session (SADS-L) and diagnostic system (RDC) as those of the London sample. Accuracy of data entries into the SADS-L was ensured by using medical case note information and all other available records. The information on all cases was reviewed by a second psychiatrist who confirmed the RDC diagnoses. All cases were diagnosed as having schizophrenia according to the probable level of the RDC. The Scottish normal control subjects were also interviewed with the SADS-L and selected on the basis of not having a family history of schizophrenia, alcoholism, or bipolar disorder and for having no past or present personal history of any RDC-defined mental disorder. Ancestry was checked and all subjects were of white English, Irish, Scottish, or Welsh origin. The subgroups of research subjects with schizophrenia and controls employed for the MRI study were all selected only from London-based schizophrenic or control samples, and the imaging research was carried out with approval from the National Health Service multicenter research ethics committee, the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint Research Ethics Committee (University College London Hospitals National Health Service Trust), and the University College London Hospitals ethics committee.

GENETIC METHODS

Novel CA dinucleotide repeat polymorphisms were identified by screening genomic DNA close to the marker D8S261. Primers to amplify these repeats were designed using the Primer3 software program.24 The 3 (CA)n repeat markers were D8S261, D8S2615, and D8S2616. Their repeat fragment sizes, respective allele numbers, and heterozygosities are listed at http://www.gdb.org (the Genome Database). Genotyping methods for the single nucleotide polymorphism markers rs916550, rs412750, rs454755, rs208753, rs3780103, rs6991775, rs445422, rs412750, rs13276297, rs3214087, and rs370429 are at Ensembl, http://www.ensembl.org; the University of California, Santa Cruz, genome browser, http://www.genome.ucsc.edu/; and the Single Nucleotide Polymorphism database at the National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/projects/SNP/. Polymerase chain reaction conditions for amplification were as previously described.25 The familial test of association was computed with the ETDT.23 The simulation approach. Subtests of the CLUMP program were the control samples. All

VOXEL-BASED MORPHOMETRY

There were 2 patient groups. One group with schizophrenia (SZ8) was selected on the basis that they had inherited marker alleles from any 2 markers that were associated with schizophrenia. This group comprised 14 subjects (mean±SD age, 40.9±8.4 years; 6 male and 8 female patients). A second patient group also had schizophrenia (SZ0) but had not inher-
We incorporated an additional step to correct for volume changes introduced during nonlinear spatial transformations. This involved modulating the image partitions (e.g., the gray and white matter segments) by the Jacobian determinants derived from the spatial normalization step. In effect, an analysis of modulated data tests for regional differences in the absolute amount (volume) of gray/white matter whereas an analysis of unmodulated data tests for regional differences in concentration of gray/white matter in the voxel. Finally, all the normalized, segmented, modulated, and unmodulated images were smoothed with a 12-mm, full width at half maximum, isotropic Gaussian kernel. This conditions the residuals to conform more closely to the Gaussian random field model underlying the statistical process used for adjusting P values.\(^1\,\,\,2\)

Global measures of gray matter, white matter, and CSF volumes were calculated from modulated segmented images to test for group differences in overall tissue compartment volumes. Second, separate analytic design matrices were constructed to test for regional differences in gray and white matter tissue compartments between patients with schizophrenia and control subjects. In these analyses, subject data were entered as 4 groups (SZ8, SZ0, CON8, CON0). Age, sex, and total intracranial tissue volume (derived from the sum of the global measures of compartmental volumes) were entered into these analyses as confounding covariates. Thus, we tested for between-group differences that were independent of age, sex, and overall head size.

Statistical parametric maps were constructed to test for morphological differences between subjects with schizophrenia and controls. Data are reported at a significance of \(P<.05\), corrected for multiple comparisons using the false discovery rate correction.\(^4\) Note that all these regional effects survived a voxelwise threshold significance of \(P<.001\), uncorrected for multiple comparisons. We tested first for global differences in tissue compartment volumes. We then tested for regional differences in gray and white matter volumes between all patients with schizophrenia and controls to study the main effect of schizophrenia. Subsequently, we tested for group differences that were independent of age, sex, and overall head size.

RESULTS

GENETIC ASSOCIATION

We reanalyzed the data from our previously published whole genome scan in schizophrenia families using markers on chromosome 8 that were approximately 10 centimorgans apart. The test of association (ETDT) in this family sample between alleles of the marker D8S261 and schizophrenia, but not the other chromosome 8 markers, was found to be significant after taking multiple alleles into account \((P = .006, \text{ data not shown})\). We followed this up in the case-control sample and found that D8S261 was also significantly associated with schizophrenia \((P = .009)\). The linkage disequilibrium relationships between these markers computed with GENECOUNTING/LDPAIRS as in Figure 1 showed that all markers associated with schizophrenia were in significant linkage disequilibrium \((P < .001)\) with 1 or more of the other associated markers (Figure 1). We then genotyped 12 other closely linked markers within the PCM1 gene \((\text{Figure 5})\). Three of these markers also demonstrated significant allelic association. The associated markers were rs445422, rs13276297, and rs370429. The markers D8S2616 and rs3214087 both showed a trend toward association with \(P\) values of .07. Markers D8S2615, rs916550, rs412750, rs457455, rs208753, rs3780103, and rs6991775 did not show evidence for association with schizophrenia. The markers showing positive tests of single marker allelic association with schizophrenia are in Table 1. These markers were combined into haplotypes and these showed significant association with schizophrenia as in Table 2. All the associated markers were within or very close to the PCM1 gene and no other genes in the region could be implicated (Figure 5). Empirically derived significance values for these tests of allelic and haplotypic association are in Table 1 and Table 2.

Two nonoverlapping sets of 3 markers were combined into separate haplotypes (PCM1A1/2 and PCM1B1/2) and all 4 haplotypes showed significant association with schizophrenia (Table 2). Replication of evidence for association with the 3 positive markers was attempted in 2 independent samples. The US replication sample of 100 parent offspring trios including 100 cases of schizophrenia showed that the same dinucleotide allele \((142 \text{ base pairs [bp]})\) at the D8S261 locus was also associated with schizophrenia \((P = .03)\) as in Table 2. A Scottish sample of 200 cases and 200 controls failed to show any significant association between schizophrenia and 3 microsatellite markers \((\text{D8S2615, D8S261, D8S2616})\) near or within the PCM1 locus. Other markers flanking the PCM1 locus, including D8S2615, rs916550, rs412750, rs457455, rs208753, rs3780103, and rs6991775, showed no individual allelic association with schizophrenia in the London sample. However, markers rs457455, rs3780103, rs6991775, and rs6991775 all showed association with schizophrenia when combined into the haplotypes PCM1A1/2 and PCM1B1/2.
The research subjects with schizophrenia were selected for the MRI study on the basis that they had inherited alleles associated with schizophrenia at any 2 of the marker loci previously shown to be associated with schizophrenia. These were allele length 142 bp at marker D8S261, allele length 205 bp at marker D8S2616, allele T at rs445422, allele T at rs13276297, and allele A at rs370429. In analyses of structural MRI scans, patients with schizophrenia had significantly reduced total and relative gray matter volumes compared with controls, and this was apparent in both PCMI-associated cases (SZ8) and non–PCMI-associated (SZ0) patients (mean±SD volumes; controls, 715.8±69 mL; SZ8, 644±63 mL; SZ0, 677±75 mL; $F_2=5.2$, $P<.05$). Mean±SD percentage gray of white matter volume were controls, 63.9%±0.02%; SZ8, 62.8%±0.01%; and SZ0, 63.3±0.02%; $F_2=5.2$, $P<.05$. Patients with schizophrenia and controls did not differ in total white matter volume ($F_2=1.1$, $P=.25$) or CSF volume ($F_2=3.0$, $P=.097$). Within the schizophrenic group, we observed no significant difference between SZ8 and SZ0 patient groups in these measures of global tissue volumes.

**BRAIN MORPHOLOGY**

We examined for group differences in regional brain morphology in separate analyses of gray matter controlling for total intracranial volume, age, and sex. Compared with controls, patients with schizophrenia (combined SZ8 and SZ0 groups) showed a distributed pattern of gray matter volume deficit, encompassing right dorsolateral prefrontal cortex, bilateral dorsomedial and orbitomedial prefrontal cortices, insulae, temporal poles, medial thalamus, posterior hippocampi, inferior temporal (fusiform) cortices, occipital (striate) cortex, and lateral cerebellar hemispheres (Figure 2). Descriptive analysis of trends identified only 1 region in patients with schizophrenia showing a trend in regional gray matter volume augmentation relative to controls. This cluster was located within the primary visual cortex (−3, −59, 3; $P<.001$, uncorrected, $Z=3.69$).

We identified differences between SZ8 and SZ0 patients in the relative distribution of gray matter within those brain areas showing a main effect of diagnosis. Patients in the SZ8 group compared with matched controls had relatively lower gray matter volume, shown in red in Figure 3, located maximally in the bilateral orbitofrontal cortices ($P<.05$, corrected). In contrast, SZ0 patients had significantly less gray matter, shown in blue in Figure 4, compared with matched controls in temporal poles, particularly on the left, medial temporal lobe (anterior hippocampus), right inferior temporal lobe, medial thalamus, right cerebellum, and dorsomedial prefrontal cortex ($P<.05$, corrected for main effect of schizophrenia) (Figure 3).

We formally tested for morphometric differences between SZ8 and SZ0 in the pattern of regional gray matter decreases in an interaction analysis in which differences between SZ8 and matched controls were compared with differences between SZ0 and matched controls. The search volume for this analysis, as shown in Figure 4, was constrained using a mask derived from the main effect of diagnosis. Grey matter volume was significantly lower in SZ8 compared with SZ0 patients within medial orbitofrontal cortex bilaterally (x, y, z coordinates [millimeters] from anterior commissure: −7.5, 34.5, −22.5 and 6, 34, −28; $P<.05$, corrected). Patients in the SZ0 group showed a significant paucity of gray matter compared with SZ8 patients in the left hippocampus (−27, −12, −1), the bilateral temporal poles (−37, 21, −42 and 39, 19.5, −34.5),

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**Figure 2.** Gray matter differences associated with main effect of schizophrenia. The figure depicts the anatomical locations in red-yellow of significantly ($P<.05$, corrected) less gray matter volume in groups of patients with schizophrenia (SZ8 and SZ0) relative to matched controls (CON8 and CON0). Data representing group differences are plotted on sagittal (A) and axial (B) sections of a normalized template brain. Distance in millimeters lateral (x coordinate) to the anterior commissure and vertical (z coordinate) to the anterior commissure/posterior commissure line are given at the bottom left corners of these sections. Marked regional foci of gray matter differences are marked as follows: ac indicates anterior cingulate; dmpfc, dorsal medial prefrontal cortex; ofc, orbitofrontal cortex; th, thalamus; oc, occipital cortex; hc, hippocampus; ins, insula; and itc, inferior temporal lobe.
and the right dorsolateral prefrontal cortex (30, 46.5, 19.5) (P<.05, corrected) (Figure 1).

**COMMENT**

The interpretation of the level of statistical significance in the allelic association studies should consider both multiple alleles and multiple markers. In the study of brain morphology, the number of statistical tests carried out should be considered. In the single marker tests of allelic association, we used empirical tests of significance, which do not need further correction for multiple alleles. For the global tests of haplotypic association with schizophrenia, further correction for both multiple alleles and multiple markers are not required because the permutation test significance takes both into account. The finding of extended transmission disequilibrium between D8S261 with schizophrenia in the family linkage sample was the reason to select this and other markers within the PCM1 gene for genotyping in the case-control sample. In addition, the marker D8S261 had been independently studied and implicated in schizophrenia in the National Institute of Mental Health trio sample and not as a result of the positive ETDT in the UK family sample or positive association in the UK case-control sample. The other markers showing association in the UK case-control sample were selected for being in linkage disequilibrium with each other. Taking into account that D8S261 had already been implicated in schizophrenia in prior studies and the fact that the additional markers genotyped were not independent tests of association, it can be argued that further corrections of significance values for testing association with multiple markers in the UK case-control sample are not needed.

Our results demonstrate that a chromosomal locus showing replicated evidence of linkage with schizophrenia in several family studies can be identified by observing linkage disequilibrium between markers and schizophrenia in a case-control association sample in which the cases have not been selected for a positive family history of schizophrenia.
Figure 4. Between-group gray matter differences in orbitofrontal cortex and temporal pole. A, Orbitofrontal gray matter volume deficit in the group of patients with PCM1 alleles associated with chromosome 8 schizophrenia (SZ8), showing involvement of the olfactory gyrus. Adjacent and to the right are plotted the effect sizes of the different parameter estimates. This is given in arbitrary units proportional to the percentage difference in volume-adjusted signal. It is the relative differences rather than the scaling of these values that is the key metric. A, There was relative reduction in orbitofrontal gray matter (at location x, y, z; −7.5, 34.5, −22.5) in both the SZ8 group and the group of patients with schizophrenia with none of the chromosome 8 PCM1 alleles (SZ0). However, the difference was marked for the SZ8 patients, reaching significance for an interaction, (CON8−SZ8)−(CON0−SZ0). B, Temporal pole gray matter volume deficit in SZ0 patients. Locations of gray matter differences between SZ0 patients and other subjects are illustrated on sagittal and axial sections of a template brain. Adjacent is a plot of parameter estimates for the peak of these differences at the left temporal pole. The greater difference in temporal lobe gray matter volume existed between SZ0 and CON0 subjects. The interaction, (CON0−SZ8)−(CON8−SZ8), was significant. CON8 indicates research subjects who are normal controls matched to the SZ8 cases; CON0, research subjects who are normal controls matched to the SZ0 cases.
nia. The fact that we were able to detect linkage disequilibrium in a sample of 450 cases of schizophrenia and find evidence in support of this in an independent sample of 100 US trio cases suggests that the chromosome 8p22 schizophrenia susceptibility alleles may be present in most European populations. As with other reported genetic associations with schizophrenia, it is clear that only a small fraction of research subjects with schizophrenia have a genetic susceptibility from the PCM1 gene locus. This confirms the general findings of linkage studies, which are explicable only by assuming the presence of extensive locus heterogeneity for genes involved in schizophrenia. We attribute the failure to find association in the Scottish sample as being due to a different admixture of genetic subtypes in Scotland or possibly because only a limited set of 3 markers could be genotyped in this sample. The UK case-control sample employed for this study has previously been shown to exhibit allelic association to the epsin 4 gene on chromosome 5, which encodes enthoprotin, a clathrin-associated protein involved in vesicle endocytosis in the brain and elsewhere.20

The gene PCM1 is involved in the maintenance of centrosome integrity and the regulation of the microtubule cytoskeleton. Its protein structure bears similarities to the structural myosin proteins, which are microtubule-associated proteins involved in axon guidance, synaptogenesis, functioning of the synapse, and intracellular transport along axons and dendrites.28 It is of note that a different gene disrupted in schizophrenia (DISC1), known to straddle a translocation breakpoint on chromosome 1 that cosegregates with schizophrenia and other types of psychiatric disorder in a single large Scottish pedigree,29 also has similarities to structural proteins such as myosins. It seems likely that PCM1 has a role in the development of the nervous system and neuronal activity. For example, it is known that PCM1 interacts with the brain-specific protein huntingtin-associated protein 1 (HAP1) that binds variably to huntingtin in relation to the number of glutamine repeat lengths present. PCM1 also interacts with cytoskeletal, vesicular, and motor proteins to mediate interactions among these different molecules.28

Our neuroimaging findings further validate the genetic data in so far as we demonstrate distinct differences in regional gray matter abnormalities in patients with chromosome 8p22 PCM1–associated schizophrenia (SZ8) compared with non-chromosome 8 PCM1–associated cases (SZ0). The significance levels reported were corrected for multiple tests and are therefore con-

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### Table 1. Single Marker Allelic Associations With Schizophrenia in the London-Based Case-Control Sample

<table>
<thead>
<tr>
<th>Marker</th>
<th>Distance to Next Marker, bp</th>
<th>Variant Bases or Marker Fragment Sizes With Allele Frequencies Below</th>
<th>CLUMP Empirical P Value</th>
<th>( \chi^2 )</th>
<th>( P ) Value</th>
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<td>25 753</td>
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Abbreviation: bp, base pair.
*Denotes most likely alleles to be associated with schizophrenia in each haplotype.
†Permutation test empirical \( P \) value.

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### Figure 5. Map of putative chromosome 8p21-22 schizophrenia susceptibility loci on 8p and diagram of the genomic structure of PCM1 showing the relative positions of exons, introns, and genetic markers. Distances are shown in megabases of DNA. PCM1 indicates pericentriolar material 1; PPP3CC, calcineurin; DRP2, dihydropyrimidinase-related protein 2; FZD3, frizzled; and NRG1, neuregulin.

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sensitive. A larger sample size, which would reduce the standard error and increase confidence in the results, would require another 450 cases of schizophrenia to be sampled and genotyped at the PCM1 locus to test for association. Therefore, replication in an independent sample is desirable.

Our evidence suggests that brain regions showing the greatest differences between the schizophrenic groups are subsumed within a wider matrix of regional brain abnormalities that span other genetic susceptibilities to schizophrenia. Nevertheless, the peak locations of reduced gray matter volume in SZ8 and SZ0 groups predict important patterns of neuropsychological deficits or vulnerability that can perhaps inform etiopathological mechanisms. Selective gray matter deficit in orbitofrontal cortex, observed in the SZ8 group, is likely to preferentially compromise neural mechanisms supporting reward-related processing and motivational behaviors. In such cases, symptomatology of the patient may be biased toward affective and behavioral features.

In contrast, patients with non–chromosome 8 linkage to 8p21-22 had significantly more affective deterioration, poorer outcome, more thought disorder, and fewer depressive symptoms than affected individuals from non–linked families.30

Further collections of genotyped patients are now needed to confirm the PCM1 8p22 allelic association with schizophrenia and to replicate the finding of brain morphology changes associated with PCM1–associated marker alleles. Modest but statistically significant levels for PCM1 marker association with schizophrenia have been found in this study. This is compatible with the fact that linkage studies have already proven heterogeneous.

### Table 2. Haplotypic Associations With Schizophrenia

<table>
<thead>
<tr>
<th>Haplotype Label</th>
<th>No. of Marker Loci</th>
<th>Alleles Increased in Haplotypes Showing Association With Schizophrenia</th>
<th>Frequency in Cases, %</th>
<th>Frequency in Controls, %</th>
<th>Global Log Likelihood Ratio χ²</th>
<th>Permutation Significance</th>
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<tbody>
<tr>
<td>PCM1A1</td>
<td>3 Marker</td>
<td>rs445422 allele C, 87366_66 allele T, rs370429 allele G*</td>
<td>29</td>
<td>17</td>
<td>14.66</td>
<td>.003</td>
</tr>
<tr>
<td>PCM1A2</td>
<td>3 Marker</td>
<td>rs445422 allele T, 87366_66 allele C, rs370429 allele A*</td>
<td>23</td>
<td>12</td>
<td>14.66</td>
<td>.003</td>
</tr>
<tr>
<td>PCM1B1</td>
<td>3 Marker</td>
<td>rs454755 allele A, rs3780103 allele G, rs6991775 allele A*</td>
<td>40</td>
<td>21</td>
<td>18.28</td>
<td>.002</td>
</tr>
<tr>
<td>PCM1B2</td>
<td>3 Marker</td>
<td>rs454755 allele G, rs3780103 allele A, rs6991775 allele A*</td>
<td>7</td>
<td>0</td>
<td>14.66</td>
<td>.003</td>
</tr>
<tr>
<td>PCM1C1</td>
<td>4 Marker</td>
<td>rs454755 allele A, 87366_66 allele C, rs3780103 allele G, rs6991775 allele A*</td>
<td>1</td>
<td>0</td>
<td>28.72</td>
<td>.002</td>
</tr>
<tr>
<td>PCM1C2</td>
<td>5 Marker</td>
<td>rs454755 allele G, 87366_66 allele C, rs3780103 allele A, rs6991775 allele A*</td>
<td>5</td>
<td>2</td>
<td>14.66</td>
<td>.003</td>
</tr>
</tbody>
</table>

*Denotes most likely alleles to be associated with schizophrenia in each haplotype.

### Table 3. Transmission of Individual Alleles at Marker D8S261 and TDT Analysis in the US Trio Sample*

<table>
<thead>
<tr>
<th>Transmission</th>
<th>Dinucleotide Repeat Lengths and Allele Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele counts transmitted</td>
<td>38</td>
</tr>
<tr>
<td>Allele counts not transmitted</td>
<td>11</td>
</tr>
</tbody>
</table>

Abbreviations: bp, base pairs; TDT, transmission disequilibrium test.

*χ² for allele-wise TDT = 18.88, P = .03. χ² for genotype-wise TDT = 43.21, P = .01.
ity of linkage in schizophrenia. The linkage studies can only be explicable if one assumes that no single schizophrenia susceptibility gene has an effect in more than 5% to 10% of families. By inference, this would also apply to case-control samples as well. High levels of significance really would not be expected from a case-control sample if only 5% to 10% of cases shared a common genetic susceptibility.

Because some of the single nucleotide polymorphism marker alleles associated with schizophrenia are at both low and high frequencies, sufficient numbers of cases and controls are needed to obtain a good chance of replication. Assuming complete linkage disequilibrium between markers and disease alleles for a significance of P<.05 and a power of 80% of replicating the result (ie, 1 in 4) with a low-frequency allele present in 5% of controls and 10% of cases, then 600 cases and 600 controls should be enough. For a high-frequency disease-associated allele present in 50% of cases and in 43% of controls, then 1300 cases and 1300 controls are needed. We can now sequence DNA from cases who have inherited PCMI gene marker alleles associated with schizophrenia to define the precise etiological base pair changes or mutations increasing susceptibility to schizophrenia. If these can be found, then exactly how PCMI shapes regional brain structure and function can be understood. The alternative splicing of PCMI messenger RNA, interaction of PCMI with other proteins, posttranslational modification, phosphorylation, and glycosylation may all play a role in mediating genetic effects on the disease.

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