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Physiogenomic Analysis of Localized fMRI Brain Activity in Schizophrenia

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Abstract

The search for genetic factors associated with disease is complicated by the complexity of the biological pathways linking genotype and phenotype. This analytical complexity is particularly concerning in diseases historically lacking reliable diagnostic biological markers, such as schizophrenia and other mental disorders. We investigate the use of functional magnetic resonance imaging (fMRI) as an intermediate phenotype (endophenotype) to identify physiogenomic associations to schizophrenia. We screened 99 subjects, 30 subjects diagnosed with schizophrenia, 13 unaffected relatives of schizophrenia patients, and 56 unrelated controls, for gene polymorphisms associated with fMRI activation patterns at two locations in temporal and frontal lobes previously implied in schizophrenia. A total of 22 single nucleotide polymorphisms (SNPs) in 15 genes from the dopamine and serotonin neurotransmission pathways were genotyped in all subjects. We identified three SNPs in genes that are significantly associated with fMRI activity. SNPs of the dopamine beta-hydroxylase (DBH) gene and of the dopamine receptor D4 (DRD4) were associated with activity in the temporal and frontal lobes, respectively. One SNP of serotonin-3A receptor (HTR3A) was associated with temporal lobe activity. The results of this study support the physiogenomic analysis of neuroimaging data to discover associations between genotype and disease-related phenotypes.

Keywords

Schizophrenia; Genetics; Auditory oddball; Functional MRI; Physiogenomics; Biomarkers; Endophenotypes; Dopamine receptors; Serotonin receptors

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INTRODUCTION

Brain imaging techniques such as positron emission tomography, magnetic resonance imaging (MRI), magneto-encephalography, and electro-encephalography are being applied increasingly to study the structure, function, and psychopathology of the human brain.[94,95,97,101,110] For psychiatric disorders, the correct phenotype for study has traditionally been based on clinician observation supplemented by the patient's subjective report of mental state. More recently, some have argued that biological factors such as electrophysiologic measures, or brain structural or functional determinations associated with the illness should be added to the conventional disease phenotype.[106] Such biologically based measures that are reliably associated with the illness have been termed "biomarkers."[80,102] When such biomarkers are identified with greater frequency in unaffected, close relatives of persons with the illness than in the general population, they have been termed intermediate phenotypes or "endophenotypes."[47] Genetics has also been of considerable interest in the study of schizophrenia, which has been shown to have a strong inherited component in twin studies[54]; several candidate risk genes have been identified.[40]

Recent results suggest that in schizophrenia disturbances in working memory and associated prefrontal cortex activation may be two genetically influenced markers for vulnerability to schizophrenia[8,35,68,93] that may be endophenotypes as well as biomarkers.[13] Multiple studies report that certain brain regions such as anterior cingulate cortex and prefrontal cortex may be closely related to specific dysfunctions seen in schizophrenia.[1,44] However, relatively little is known about the genetics of these dysfunctions, although their potential in the search for genetic associations has been recognized.[34,69,108]

There is substantial evidence that schizophrenia is likely to be a complex genetic disorder, with multiple risk genes of individually weak effect.[79] Therefore, a multivariate approach, capable of extracting hidden cross-information from a larger number of genes, has the potential to uncover influences of multiple genetic factors on the functions of both healthy and disordered brains. Most genetic risk studies focus on particular genes or polymorphisms of interest and examine the univariate relationship between a genotype and a phenotype. For example, the association of the G72/G30 locus with schizophrenia and bipolar disorder has been reported in several studies.[22,36] Other genes and polymorphisms studied in schizophrenia include a brain-derived neurotrophic factor (BDNF) prodomain polymorphism,[2,41,72] Catechol-O-methyl-transferase (COMT),[42,71,75] gamma aminobutyric acid (GABA),[38,55,59,112] histidine triad nucleotide-binding protein (HINT1) and cytosolic malate dehydrogenase (MDH1).[3,16,109] Some researchers have presented data derived from multimodal imaging or combined data from both imaging and other modalities, including genomic imaging).[31,39] Further developments to extend and implement multimodal aspects of the disease are needed.

The recent availability of the human genome sequence[53] and its variability[96,107] together with the development of massively parallel genotyping has led to a rush to search for genetic determinants of physiological variability, especially as it pertains to human diseases. Typically, a population of patients is assessed for the trait of interest and compared to a control cohort, and DNA samples are taken for genotyping. The samples are genotyped at selected single nucleotide polymorphisms (SNPs) and statistical analysis is performed to determine if the genotypes are significantly associated with the trait.

The results of these population studies have been inconsistent. Often, results are published that do not later replicate in other studies.[14,19,23,32,82,91] There are multiple factors that render it difficult for these studies to produce reliable results, such as the high rate of false positives due to multiple testing,[73] population structure, [64] and the presence of confounding factors or limitations in the clinical data.

We have recently developed a biomedical engineering approach, termed "physiogenomics,"[43,89] to address the inherent limitations of genetic associations. Physiogenomics is a medical application of sensitivity analysis. Sensitivity analysis is the study of how variation of system components contributes to variation in output response to a given input.[92] Physiogenomics utilizes the variability in genes as system components, measured by SNPs, and determines how the SNP frequency among individuals relates to the variability in physiologic characteristics, the output. Physiogenomics recognizes that in most cases no single polymorphism will provide useful information about a phenotype of interest, and advances the concept of ensembles of SNP markers that together with a predictive algorithm can explain a large part of the variation in response. Physiogenomic analysis takes advantage of the concept of systems biology by including biomarkers in the analysis to bridge the gulf of complexity between gene polymorphisms and clinical observations. In this study, we focus on the use of biomarkers as endophenotpyes, while applying standard methods for SNP association testing.

We used neuronal activity measured by functional magnetic resonance imaging (fMRI) during the performance of an auditory oddball task, already shown to be pertinent to schizophrenia pathophysiology,[10,50] as a biomarker. In this task, infrequent novel target stimuli must be detected among both novel and frequent standard background stimuli. Electrophysiologic event-related potentials (ERP) have been used to study aspects of the orienting response in for many years. There, the novel stimuli are associated with a sequence of electrical components the most prominent of which is a large broadly distributed positive wave termed the 'P3' or 'P300.' The P3 elicited by novel and target stimuli likely represent processes involving attention capture, allocation of cognitive resources, and contextual updating. These are all components of the orienting response, which is abnormal in schizophrenia.[65]

Event-related fMRI has made it possible to measure the hemodynamic response for distinct events.[50,77,84] Prior investigators have performed such studies of brain activity associated with processing low-probability sensory stimuli. McCarthy *et al*. found that oddball visual stimuli elicited activation in the middle frontal gyrus, inferior parietal lobule (IPL), and posterior singulate cortex (PCC).[66] Menon *et al*. found that auditory target stimuli generated activity in IPL,[67] and a large-scale study of auditory oddball processing in healthy controls[50] illuminated a large-scale circuit including the previously mentioned regions. Several recent fMRI studies attempt to elucidate brain activity elicited by stimuli producing the P3b,[9,15,48,49,58,66,67,78,100] which implicates multiple heteromodal cortical and other brain regions. The auditory oddball fMRI probe is thus attractive as a general activator of a widespread network, requiring little effort from schizophrenia patients, whose task performance accuracy is generally unimpaired, but where task-related activation is abnormal.[33] Thus, the task is now well explored in patients (and their relatives) as a putative biological marker and endophenotype.[11,12]

In addition to dynamic techniques such as ERP and fMRI, MRI-derived structural endophenotypes have also been studied. While several reviews have been optimistic, e.g., Keshavan *et al*.,[46] other recent publications have yielded disappointing results, e.g., Goldman *et al*.[37] Thus, we focus here on functional MRI as an event-related methodology that can also provide information regarding functional connectivity among distant brain regions.

Our goal was to discover physiogenomic associations between genes related to dopamine and serotonin neurotransmission and fMRI of locations in temporal and frontal lobes previously associated with schizophrenia, [11,56] as a demonstration of the potential of our method to illuminate the genetic basis of this multigenic disease.

METHODS AND MATERIALS

Participants

Participants were recruited via advertisement, presentations at local universities and clinics and by word-of-mouth. A total of 99 participants, including 30 patients with schizophrenia, 13 unaffected first-degree relatives of patients with schizophrenia, and 56 healthy controls were involved. Prior to inclusion in the study, control participants were screened to ensure they were free from DSMIV Axis I or Axis II psychopathology (assessed using the SCID[98]) and also interviewed to determine that there was no history of major mental illness in any first-degree relatives. Patients met criteria for schizophrenia in the DSM-IV on the basis of a structured clinical interview[28] and review of the case file. None of the subjects met criteria for alcohol/drug abuse or dependence. The study was approved by the Hartford Hospital IRB and all subjects gave written informed consent.

There was no significant between-group difference in age (controls, 47 ± 16 years, range 21–84; patients, 41 ± 10 years, range 20–55; relatives, 49 ± 19 years, range 20–87). There were 30 female and 26 male participants in the 56 healthy controls, 7 female and 23 male patients, and 5 female and 8 male relatives. A total of 80 subjects described themselves as Caucasian, 10 as African American, 4 as Hispanic, 2 as Asian, 2 as mixed, and 1 was unspecified. Full scale IQ scores for the groups evaluated from the National Adult Reading Test (NART)[6,7] were 109.8 ± 7.5 for controls, 101.8 ± 11.3 for patients, and 100.9 ± 7.8 for relatives (only 45 controls, 27 patients, and 12 relatives had NART scores recorded).

fMRI Data Collection

The fMRI data were collected during performance of an auditory oddball task,[48,49] which consists of detecting an infrequent sound within a series of frequent sounds. Auditory stimuli were presented to each participant by a computer stimulus presentation system via earphones. The standard stimulus was a 500-Hz tone, the target stimulus was a 1000-Hz tone, and the novel stimuli consisted of non-repeating random digital noises (e.g., tone sweeps, whistles). The full description of task design is available elsewhere.[51] The participants were instructed to respond as quickly and accurately as possible with their right index finger every time they heard the target stimulus.

Scans were acquired at the Olin Neuropsychiatry Research Center at the Institute of Living on a Siemens Allegra 3T dedicated head MRI scanner equipped with 40mT/m gradients and a standard quadrature head coil. The functional scans were acquired using gradient-echo echo-planar-imaging with the following parameters: repeat time (TR) = 1.50 s, echo time (TE) = 27 ms, field of view = 24 cm, acquisition matrix = 64 × 64, flip angle = 70°, voxel size = 3.75 × 3.75 × 4 mm^3, slice thickness = 4 mm, gap = 1 mm, 29 slices, ascending acquisition.

fMRI Data Preprocessing

Six “dummy” scans were performed at the beginning to allow for longitudinal equilibrium, after which the paradigm was automatically triggered to start by the scanner. Images were realigned using INRI-align —a motion correction algorithm unbiased by local signal changes.[29] Data were preprocessed using the software package SPM2 (http://www.fil.ion.ucl.ac.uk/spm/). Data were spatially normalized into the standard Montreal Neurological Institute space,[30] re-sliced to 3 mm^3, and spatially smoothed with a 10 mm^3 Gaussian kernel. Data for each participant were analyzed by a multiple regression incorporating regressors for the novel, target, and standard and their temporal derivatives plus an intercept term. The target- vs.- standard contrast images were used in this study.

We defined two large regions of interest based on previous findings of schizophrenia-related abnormalities in the frontal and temporal lobes.[11,56] The regions were BA 10/46 in the lateral frontal lobe (dorsolateral prefrontal cortex), and superior and middle temporal gyri (STG/MTG) in the temporal lobe, as defined in the wfu_pickatlas toolbox (http://www.fmri.wfubmc.edu/).[62] A voxel-wise two-sample *t*-test between patients and controls was performed on the target-related contrast images, and the voxel of highest discrimination between patients and controls (maximal voxel) was located in each region. Frontal and temporal endophenotype values were obtained by extracting the PSC for each patient from the respective maximal voxels. Figure 1 shows the location of the two regions and also indicates the maximal voxels which were used to generate the PSC phenotypes.

Candidate Gene Selection

Table 1 shows the candidate genes selected for this study. The genes were selected from the PG Array, a SNP Array consisting of 384 SNPs from 222 genes that has been designed and tested at Genomas as a product[85] and service. The array covers genes from a variety of metabolic processes and neurotransmitter axes. For this study, we focused on 16 genes related to dopaminergic and serotonergic neurotransmission, including dopamine beta-hydroxylase (DBH), dopamine receptors D1, D2, D3, D4, D5, dopamine transporter, 5a-hydroxytryptamine receptors 1A, 1D, 2A, 2C, 3A, 3B, 5A, 6, and 7 and the serotonin transporter solute carrier family 6 A4, which are represented on the array by 24 SNPs.

Laboratory Analysis

A blood sample was obtained for each subject and DNA extracted. Genotyping was performed using the Illumina BeadArray™ platformand the GoldenGate™ assay.[25,76] Table 2 lists the assay information and observed allele frequencies for the SNPs used in this study.

Data Analysis

Statistical analysis utilized the *R* Statistics Language and Environment.[21,27,61,81] Covariates gender, race, age, and group (affected, relative, and unaffected) were analyzed using multiple linear regression, and selected using the stepwise procedure. To test for association with SNPs, a linear regression of the covariate-adjusted residual of the PSC phenotypes was performed. SNP genotype was coded numerically according to carrier status for the minor allele: 0 for non-carriers, 1 for single carriers, and 2 for double carriers. The *F*-statistic *p*-value for the SNP variable from the analysis of variance (ANOVA) was used to evaluate the significance of association. To test the validity of the ANOVA *p*-values, we also performed an independent calculation of the *p*-values using permutation testing. The ranking of the first three SNPs was identical under permutation and ANOVA analyses (data not shown). To account for the multiple testing of 24 SNPs, we calculated adjusted *p*-values using Benjamini and Hochberg's false discovery rate (FDR) procedure.[4,5,83]

LOESS Representation

To represent the variation in SNP frequency with the response, we used a locally smoothed function of the SNP frequency as it varies with PSC to visually represent the nature of an association. LOcally wEighted Scatterplot Smooth (LOESS) is a method to smooth data using a locally weighted linear regression.[17,18,89] At each point in the LOESS curve, a quadratic polynomial is fitted to the data in the vicinity of that point. The data are weighted such that they contribute less if they are further away, according to the tricubic function

$$w_i = \left(1 - \left|\frac{x - x_i}{d(x)}\right|^3\right)^3,$$

where x is the abscissa of the point to be estimated, the x_i are the data points in the vicinity, and $d(x)$ is the maximum distance of x to the x_i.

RESULTS

Figure 2 depicts the distribution among participants of the percent signal change (PSC) in the frontal and temporal lobe areas. These distributions are approximately normal. Subset distributions are shown for the affected, relative, and unaffected groups. The PSC in both areas are markedly lower in affected vs. unaffected and related subjects. We tested the potential covariates of age, gender, race and group for association with PSC using multiple linear regression. The group variable was strongly associated with PSC both in the temporal ($p<0.001$, $R^2 = 18\%$) and frontal ($p<10^{-5}$, $R^2 = 27\%$) lobe areas. Only one other marginally significant covariate association was found: In the temporal area, male gender was found to be weakly associated with increased PSC ($p = 0.04$, $R^2 < 3\%$). Both of these covariates were adjusted for in the SNP association testing.

Table 3 lists the results of the association tests for the frontal and temporal areas. We found that SNPs in DBH (rs4531) and DRD4 (rs4987059) were significantly associated with the PSC in the temporal and frontal areas, respectively. We also found that SNP rs1150226 in HTR3A was significantly associated with the PSC in the temporal area. All remaining genes showed no significant association in either area, and no gene showed significant associations in both.

Figure 3 shows detailed representations of the physiogenomic analysis for the three significant physiogenomic associations ($p<0.05$) found for DBH, DRD4, and HTR3A. The overall distribution of PSC is shown along with the individual genotypes and a LOESS fit of the allele frequency as a function of PSC. The bell curve shows the actual distribution of the phenotype in the study population. The circles show the genotype of each subject, with the non-carriers of the minor allele at the bottom, the single-carriers in the middle, and the double-carriers at the top. The position of the circle along the abscissa indicates the PSC observed in a specific participant. The LOESS curve shows the localized frequency of the least common allele for sectors of the distribution. For SNPs with a strong association, the marker frequency will be significantly different between the high and low ends of the distribution. For example, the first panel in Fig. 3 shows the LOESS curve for SNP rs4531 of the DBH gene. The frequency of the minor allele is 5% in subjects with low PSC. In contrast, it is above 10% in subjects with high PSC, which are mostly unaffected by schizophrenia. This finding indicates a physiogenomic association between this SNP and PSC. As the frequency of the minor allele is higher in the high spectrum of PSC, correlated with absence of disease, DBH SNP rs4531 may be considered a potential protective marker for schizophrenia.

DISCUSSION

The finding that polymorphisms in the DBH and DRD4 genes are associated with schizophrenia-related fMRI phenotypes is consistent with the dopaminergic pathophysiology of the disease.[57,60,99] The agreement between our physiogenomic association results and prior genetic studies of the disease validates the endophenotype approach integrating fMRI.

SNP rs4531 codes for an amino acid change (S304A) in exon 5 of the DBH gene. DBH catalyzes the oxidative hydroxylation of dopamine to norepinephrine.[52] Plasma DBH activity

is highly heritable.[103] Polymorphisms in the DBH gene may modulate psychotic symptoms, schizophrenia risk,[20,111] and paranoid behaviors.[45]

SNP rs4987059 is non-coding and is located in the promoter region of the DRD4 gene, a region previously associated with schizophrenia.[70] D4dopamine receptors are members of the D2-like dopamine receptor family. Immunologic assays show significant densities of D4 receptors in prefrontal cortex, thalamus, hippocampus, amygdala, and globus pallidus.[26,70,74,104,105]

SNP rs1150226 is non-coding and is located in the promoter region of the HTR3A gene. The 5-HT3 receptor is a pentameric ligand-gated cation channel located in CNS regions integrating reward and anxiety control as well as emetic and pain processing.[24] The physiological role of 5-HT3 receptors encompasses responses to serotonin, dopamine, glutamate, GABA, and substance P. The novel physiogenomic association of HTR3A found here is thus plausible.

The value of the physiogenomic associations to fMRI activity elicited by the auditory oddball stimulus in the temporal and frontal lobes is that these responses themselves are abnormal in many patients with schizophrenia, thus constituting a biomarker for the disorder. The chasm from gene to disease phenotype is thus effectively bridged by fMRI. The brain's response to task performance in healthy controls activates multiple regions, which evidence deficient activity in patients with schizophrenia.[10,50] The advantages of the auditory oddball task include its well-replicated status, ease of translation from the electrophysiological to the fMRI realm, and comparably accurate performance of the task by affected and unaffected subjects. Once the physiogenomic associations to disease are validated clinically, there would not be a need to perform fMRI in response to the oddball stimulus on each individual patient. The fMRI can be superseded in routine clinical practice, but its predictive value retained by means of physiogenomics. The predictive genotypes can then be used in clinical practice without the need to gather fMRI data on patients. The ultimate diagnostic and operational advantage of robust physiogenomic associations but-tressed by an endophenotype is the direct translation of clinical information from DNA to patient management.

This study has various limitations. Due to the modest sample size, none of the associations we observed passes the $p<0.05$ significance criterion when adjusted for multiple SNP comparisons, either by Bonferroni correction or by FDR.[4,5,83] We applied physiogenomics to a phenotype determined by fMRI and computational analysis of only two localized brain regions in frontal and temporal lobes, and only using one particular task. The true promise of endophenotypes for genetic association can only be realized when multiple different endophenotypes are analyzed and the results integrated into a coherent picture of genetic determinants and the pathways leading to their clinical manifestations. Even though we were primarily interested in a particular endophenotype and its genetic underpinnings, future studies should include more detailed clinical data, such as presence or absence of auditory hallucinations. Our recruitment of affected, related, and normal individuals with regard to schizophrenia diagnosis created a cohort with variable responses to the oddball acoustic stimulus. However, the performance of the unaffected relatives resembled that of the healthy controls more than that of the affected individuals, which we attribute to more parents than siblings being recruited to the cohort of related individuals. In the near term, we plan to extend the physiogenomic analysis to case vs. control comparisons using total genome arrays and whole brain scans to approach fMRI endophenotypes from a hypothesis-free perspective.

We had priorly demonstrated the applicability of physiogenomics in human responses to drugs, diet, and exercise.[86–90] Physiogenomics has also been successfully applied in rodent animal models by others.[63] Here we demonstrated that physiogenomics also applies to sensorial stimuli, specifically acoustic patterns, as exemplified by the oddball task. Consistent with a targeted, hypothesis-driven query, we compared genes involved in dopaminergic and

serotonergic neurotransmitter systems and determined a high level of association to DBH and DRD4, which is supported by prior pathophysiological and genetic associations to schizophrenia and uncovered a new association to HTR3A. The results demonstrate the advantage of neuroimaging analysis to discover physiogenomic associations. We believe the interplay of fMRI and physiogenomics opens new vistas for biomedical engineering approaches in neuroscience.

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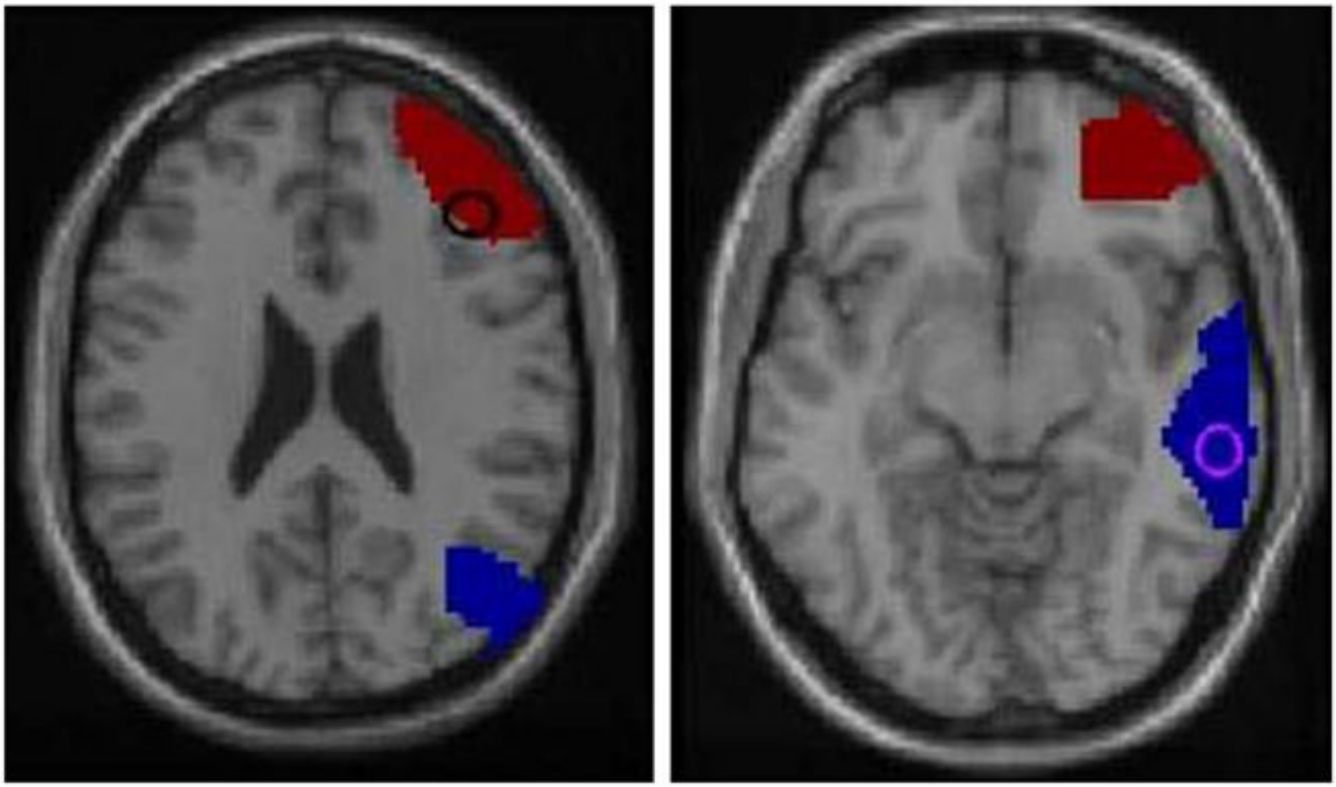

FIGURE 1.
Location of the frontal and temporal lobe regions of interest. The red region in both images is BA 10/46 in the frontal lobe, the blue region is STG/MTG in the temporal lobe. The circles indicate points of maximum discrimination between patients and controls as indicated by the voxel-wise *t*-test.

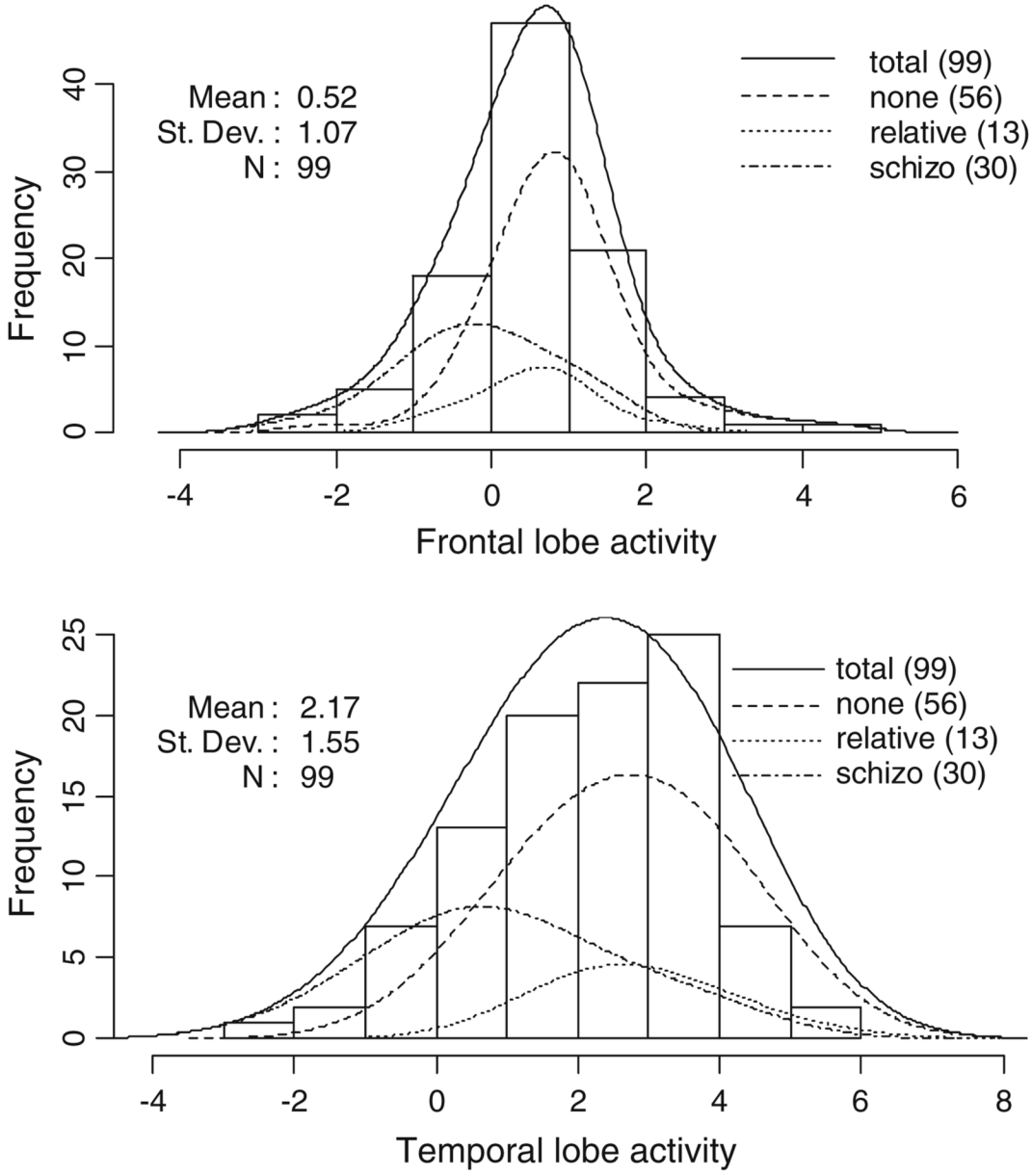

FIGURE 2.
Distribution of frontal and temporal lobe PSC for the study group. The vertical axis indicates the number (*No.*) of subjects observed within a given PSC interval on the horizontal axis.

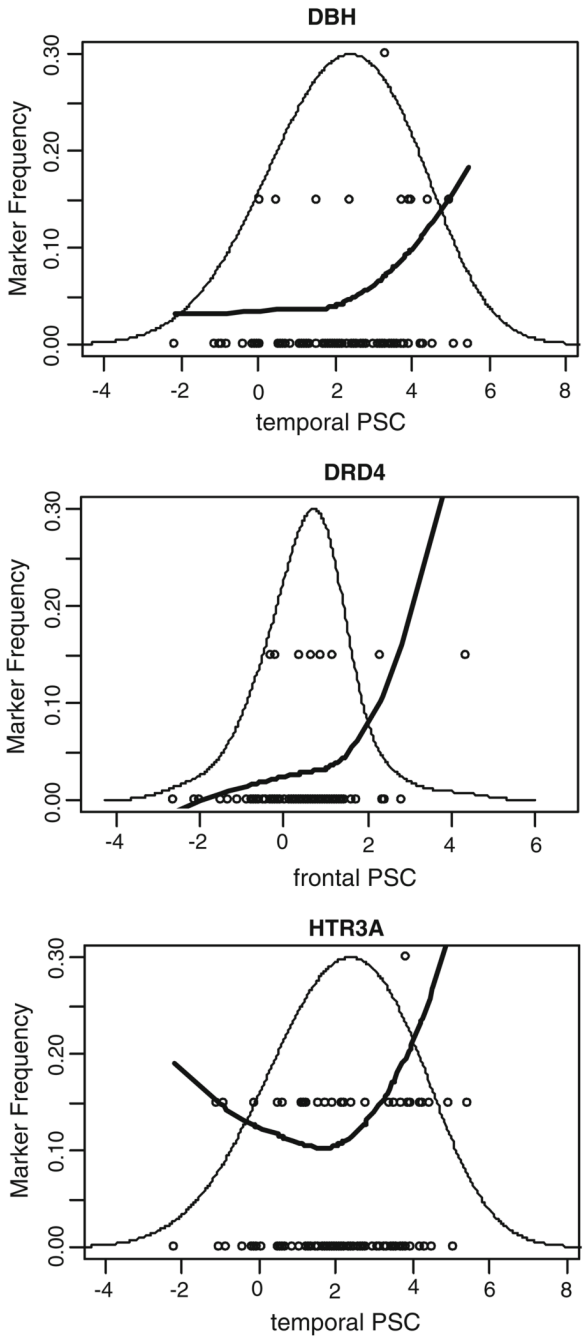

FIGURE 3.

Physiogenomic representation of significant physiogenomic associations. Individual subject genotypes (*circles*) of each SNP are overlaid on the distribution of PSC (*thin line*). Each circle represents a subject, with the horizontal axis specifying the local neuronal activity, and the vertical axis the carrier status for the minor allele: bottom, non-carriers; middle, single-carriers; top, double-carriers. A LOESS fit of the allele frequency (*thick line*) as a function of PSC is shown. The ordinate is labeled for the marker frequency (*thick line*) of the SNP denoted at the top of each panel. The ordinate scale is the same in all three panels. The ordinate scales for the genotypes (*circles*) and PSC distribution (*thin line*) are not shown. The abscissa is labeled for

neuronal activity in each panel. The abscissa scale is the same in all three panels and applies identically to marker frequency, genotypes, and neuronal activity.

TABLE 1

Genes and SNPs analyzed for associations with PSC in the frontal and temporal lobe regions of interest.

Area	Pathway	SNP	Gene	Type
Dopamine system	Metabolism	rs4531	DBH	exon 5, S304A
		rs1611115	DBH	~1 kb upstream
	Receptor	rs2298122	DRD1IP	intron 1
		rs1799978	DRD2	~500 bp upstream
		rs2471857	DRD2	intron 1
		rs167771	DRD3	intron 3
		rs167770	DRD3	intron 1
		rs9288993	DRD3	intron 3
		rs4987059	DRD4	~800 bp upstream
		rs2867383	DRD5	~2.3 kb downstream
	Transporter	rs3756450	SLC6A3	~2.5 kb upstream
Serotonin system	Receptor	rs676643	HTR1D	~200 bp upstream
		rs659734	HTR2A	intron
		rs6312	HTR2A	~600 bp upstream
		rs1150226	HTR3A	~500 bp upstream
		rs3758987	HTR3B	~200 bp upstream
		rs2276307	HTR3B	intron 6
		rs1176744	HTR3B	exon 5, S129Y
		rs1440451	HTR5A	intron 1
		rs9659997	HTR6	intron 1
		rs1935349	HTR7	intron 1
		rs1891311	HTR7	~700 bp upstream
	Transporter	rs140700	SLC6A4	intron 4
		rs2020933	SLC6A4	intron 1

TABLE 2

Assay DNA sequences for the SNPs analyzed.

Markers							HapMap	
SNP	**Gene**	**Chr**	**Maj**	**Min**	**Freq**	**Sequence**	**CEU**	**YRI**
rs4531	DBH	9	T	G	0.06	AGGACCCTGGACCCCCGAAGG[A/C]AAGGCCGGCTTCCTCTGGGT	NA	NA
rs1611115	DBH	9	T	C	0.17	CTCTCCCTCCTGTCCTCTCCC[A/G]CAAGTAGACTGAGGGCAGCT	0.17	0.12
rs2298122	DRD1IP	10	T	G	0.23	CTCCCCTCTCAGTTCAGGGCT[A/C]TCTTGGGTCCCTGCCAGCTG	0.19	0.65
rs1799978	DRD2	11	A	G	0.11	CCCAGCCTGCAATCACAGCTT[A/G]TTACTCTGGGTGTGGGTGGG	0.03	0.13
rs2471857	DRD2	11	A	G	0.19	CTTCCCAGTTGCACTAACAGA[A/G]CCTTTGATTCAGTTCAGCAA	0.17	0.17
rs167771	DRD3	3	A	G	0.28	TGCTCCAAAGTCTATCACAAT[A/G]ATCCTCTTTTCCATAAAGCC	0.17	0.87
rs167770	DRD3	3	A	G	0.29	TGGCTTCAGCTTGTAAAGCTT[A/G]GAAACATTCTGAAACAACAT	0.29	0.72
rs9288993	DRD3	3	A	G	0.04	GGCAGGTAATGATATTGTGAC[A/G]TGGAGAATGTGCACTTAGAA	0.03	0.23
rs4987059	DRD4	11	A	G	0.04	TTTGCAAGCACTTTCTCTTCT[A/G]CACGTTTGGAACCTACCCCG	NA	NA
rs2867383	DRD5	4	A	G	0.28	GCCTGTGGTCACAGAGCTCCT[A/G]AGTGGCAGAACTCAACTTGA	0.33	0.50
rs3756450	SLC6A3	5	T	C	0.11	CCTAAATGCCAAGTCGGCTTT[A/G]TTATCATTGTGGTTGCTGCT	0.16	0.50
rs676643	HTR1D	1	A	G	0.14	AGGTTCATCTTGACGCATCCT[A/G]AGCTACTTAACTTCGGTTCC	0.16	0.22
rs659734	HTR2A	13	T	C	0.07	CTGGTAGGAAATTGAACTGAA[A/G]TCATAAACGGAAAGCAGCTA	0.07	0.17
rs6312	HTR2A	13	A	G	0.06	AACAAATGTATCTCATGTGTG[A/G]ACCCTGAAGACAAATGTAAG	0.07	0.23
rs1150226	HTR3A	11	T	C	0.15	TTATGTCACCCTGGGGAGTAA[A/G]AGAATGGTCTTCCTGCTCCT	0.07	0.37
rs3758987	HTR3B	11	A	G	0.31	ACAGCCTTTACCTAAGGCAGT[A/G]CTCTTGCTGACATTCAGGAC	0.23	0.48
rs2276307	HTR3B	11	A	G	0.16	CCTTCTCTCTTGGGCCAAGGA[A/G]TTTCTGCTCTATTGCATGTT	0.17	0.08
rs1176744	HTR3B	11	T	G	0.34	ATAGTGTGGACATTGAAAGAT[A/C]CCCTGACCTTCCCTATGTTT	0.25	0.43
rs1440451	HTR5A	7	C	G	0.04	CTTGTTCATGATGAGATTATA[C/G]CTGATCTGACGTGAGAATGC	0.01	0.54
rs9659997	HTR6	1	T	C	0.46	ACAAATGCTCTGAGTCACCAC[A/G]CTGCGGCTCAGATGCTATGA	0.41	0.57
rs1935349	HTR7	10	A	G	0.17	TTATAGATTGTCCAGACATGA[A/G]CAGATCTATCACCTGACCAC	0.12	0.50
rs1891311	HTR7	10	A	G	0.07	AATGACCGGTTATACTCTTCT[A/G]TAAAGGAATCCTGGAGGTGT	0.10	0.30
rs140700	SLC6A4	17	A	G	0.09	ATCTTTCTGCCACACCACCTC[A/G]CCCTCCTTTCTCAAGGTCTT	0.10	0.04
rs2020933	SLC6A4	17	A	T	0.16	TTTTGTCCAGAAAAGTGAACC[A/T]GGTCAATGGATTATTTATGA	0.05	0.39

Also shown are chromosome location of the gene (Chr), sequence of the most common allele (major, Maj), sequence of the least common allele (minor, Min), and frequency of the minor allele in the study population (Freq), and in two HapMap populations (CEU, YRI).[41] (NA, not available).

TABLE 3

Significance levels of gene SNPs associated with fMRI activity.

Markers		*p*-Values		SNP		
SNP	**Gene**	**Frontal**	**Temporal**	**Freq**	***n***	**Type**
rs4531	DBH	0.9207	**0.0188**	0.06	98	Dopamine beta-hydroxylase (dopamine beta-monooxygenase)
rs1611115	DBH	0.1008	0.2357	0.17	93	Dopamine beta-hydroxylase (dopamine beta-monooxygenase)
rs2298122	DRD1IP	0.6815	0.4816	0.23	96	Dopamine receptor D1 interacting protein
rs1799978	DRD2	0.2300	0.5440	0.11	97	Dopamine receptor D2
rs2471857	DRD2	0.6141	0.4685	0.19	98	Dopamine receptor D2
rs167771	DRD3	0.4777	0.3505	0.28	98	Dopamine receptor D3
rs167770	DRD3	0.2801	0.5489	0.29	98	Dopamine receptor D3
rs9288993	DRD3	0.5362	0.7589	0.04	98	Dopamine receptor D3
rs4987059	DRD4	**0.0085**	0.8205	0.04	97	Dopamine receptor D4
rs2867383	DRD5	0.6244	0.5440	0.28	93	Dopamine receptor D5
rs3756450	SLC6A3	0.4102	0.5611	0.11	95	Solute carrier family 6 (neurotransmitter transporter, dopamine), member 3
rs676643	HTR1D	0.6800	0.7158	0.14	98	5-hydroxytryptamine (serotonin) receptor 1D
rs659734	HTR2A	0.4162	0.6008	0.07	98	5-hydroxytryptamine (serotonin) receptor 2A
rs6312	HTR2A	0.3919	0.3409	0.06	97	5-hydroxytryptamine (serotonin) receptor 2A
rs1150226	HTR3A	0.4706	**0.0307**	0.15	98	5-hydroxytryptamine (serotonin) receptor 3A
rs3758987	HTR3B	0.7606	0.7114	0.31	97	5-hydroxytryptamine (serotonin) receptor 3B
rs2276307	HTR3B	0.8560	0.1839	0.16	98	5-hydroxytryptamine (serotonin) receptor 3B
rs1176744	HTR3B	0.4477	0.7495	0.34	94	5-hydroxytryptamine (serotonin) receptor 3B
rs1440451	HTR5A	0.5304	0.2397	0.04	97	5-hydroxytryptamine (serotonin) receptor 5A
rs9659997	HTR6	0.6010	0.5783	0.46	98	5-hydroxytryptamine (serotonin) receptor 6
rs1935349	HTR7	0.3280	0.8064	0.17	97	5-hydroxytryptamine (serotonin) receptor 7 (adenylate cyclase-coupled)
rs1891311	HTR7	0.7008	0.4963	0.07	98	5-hydroxytryptamine (serotonin) receptor 7 (adenylate cyclase-coupled)
rs140700	SLC6A4	0.3058	0.6948	0.09	98	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4
rs2020933	SLC6A4	0.4378	0.4880	0.16	98	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4

Significant p-values (<0.05) are indicated in bold.