Analysis of *TBX1* Variation in Patients with Psychotic and Affective Disorders

Birgit H Funke,[1] Todd Lencz,[2] Christine T Finn,[1] Pamela DeRosse,[2] G David Poznik,[3] Alex M Plocik,[1] John Kane,[2] John Rogus,[3] Anil K Malhotra,[2] and Raju Kucherlapati[1]

[1]Harvard Partners Center for Genetics and Genomics, Boston, MA, USA; [2]Psychiatry Research, The Zucker Hillside Hospital, Glen Oaks, NY, USA; [3]Joslin Diabetes Center, Boston, MA, USA

A significant portion of patients with 22q11 deletion syndrome (22q11DS) develop psychiatric disorders, including schizophrenia and other psychotic and affective symptoms, and the responsible gene/s are assumed to also play a significant role in the etiology of nonsyndromic psychiatric disease. The most common psychiatric diagnosis among patients with 22q11DS is schizophrenia, thought to result from neurotransmitter imbalances and also from disturbed brain development. Several genes in the 22q11 region with known or suspected roles in neurotransmitter metabolism have been analyzed in patients with isolated schizophrenia; however, their contribution to the disease remains controversial. Haploinsufficiency of the *TBX1* gene has been shown to be sufficient to cause the core physical malformations associated with 22q11DS in mice and humans and via abnormal brain development could contribute to 22q11DS-related and isolated psychiatric disease. 22q11DS populations also have increased rates of psychiatric conditions other than schizophrenia, including mood disorders. We therefore analyzed variations at the *TBX1* locus in a cohort of 446 white patients with psychiatric disorders relevant to 22q11DS and 436 ethnically matched controls. The main diagnoses included schizophrenia (n = 226), schizoaffective disorder (n = 67), bipolar disorder (n = 82), and major depressive disorder (n = 29). We genotyped nine tag SNPs in this sample but did not observe significant differences in allele or haplotype frequencies in any of the analyzed groups (all affected, schizophrenia and schizoaffective disorder, schizophrenia alone, and bipolar disorder and major depressive disorder) compared with the control group. Based on these results we conclude that *TBX1* variation does not make a strong contribution to the genetic etiology of nonsyndromic forms of psychiatric disorders commonly seen in patients with 22q11DS.

Online address: http://www.molmed.org
doi: 10.2119/2006–00119.Funke

INTRODUCTION

22q11 Deletion syndrome (22q11DS) is the most common human microdeletion syndrome, leading to a host of developmental anomalies, which commonly include craniofacial, palatal, and conotruncal cardiovascular malformations and thymus and parathyroid hypo- or aplasia (1,2). Most deletions are 3 Mb in size and lead to reduced dosage of ~45 genes (3–5). It is estimated that more than 50% of 22q11DS patients develop psychiatric disorders, including schizophrenia, affective disorders, anxiety disorders, autism spectrum disorders, and attention deficit disorder with or without hyperactivity (6–11). Of these diagnoses, schizophrenia is the most common (12–14, reviewed in 15), and its high prevalence among patients with 22q11DS makes the syndrome the greatest known genetic risk factor for schizophrenia, second only to having an affected monozygous twin or two parents with schizophrenia. Consequently, it is thought that the gene/s responsible for the psychiatric manifestations of 22q11DS also contribute to the genetic etiology of nonsyndromic schizophrenia in the general population. A large number of population studies have implicated more than 10 of the ~45 genes in the 3-Mb region typically deleted in patients with 22q11DS in the etiology of schizophrenia (16–25, reviewed in 26). Many studies have not been replicated, however, and it is therefore highly unlikely that all of these genes play a role.

Disturbances in the metabolism of the neurotransmitters dopamine and glutamate are thought to play a fundamental role in the etiology of psychiatric disease (27–29). Two genes from the 22q11 region that are involved in the regulation of neurotransmitter levels have been extensively studied in patients with nonsyndromic schizophrenia: Proline dehydrogenase (*PRODH*) encodes a mitochondrial enzyme that catalyzes the first

Address correspondence and reprint requests to *Birgit Funke, Laboratory for Molecular Medicine, Harvard Partners Center for Genetics and Genomics, 65 Landsdowne Street, Cambridge, MA 02139. Phone: 617-768-8467; Fax: 617-768-8513; E-mail: bfunke@partners.org.*
Submitted December 31, 2006; Accepted for publication June 5, 2007.

step in the conversion of proline to the neurotransmitter glutamate (30). Mouse studies support a role in schizophrenia (31,32), and population studies have found an association of *PRODH* variants with the disorder (33,34), but overall, evidence from population studies is still controversial (35–37). Catechol-O-methyltransferase (*COMT*) inactivates catecholamine neurotransmitters (dopamine, noradrenalin, and adrenaline) by transferring a methyl group from S-adenosyl methionine (38). This role has made *COMT* a prime suspect in the genetic etiology of schizophrenia, but evidence from population studies has failed to provide definitive proof (reviewed in 26, 39). A third gene, *ZDHHC8*, was implicated on the basis of a systematic scan of the 22q11 region in patients with schizophrenia and subsequent mouse studies showing a schizophrenia-related phenotype (16,40). However, subsequent replication studies in schizophrenic patients did not support a role of *ZDHHC8* (41–45).

The neurodevelopmental model of schizophrenia is based on evidence for perturbances in brain development (46). The observation that craniofacial and limb anomalies are correlated with schizophrenia supports this hypothesis and suggests the disturbance of a shared morphogenetic mechanism (47,48). The key 22q11DS gene, *TBX1*, is involved in epithelial/mesenchymal interactions (49,50), a mechanism crucial for the development of a wide variety of organs including the forebrain, heart, face, and limbs (51,52). It is therefore conceivable that *TBX1* plays a role in brain development and thus in 22q11DS-associated as well as nonsyndromic psychiatric disease.

Several mouse studies have analyzed *Tbx1* expression in the brain and, with one exception (53), have provided convincing evidence for expression at low levels during prenatal brain development and then in a steadily increasing fashion from birth to three months (54–56). In addition, evidence supporting a role of *TBX1* in psychiatric disease comes from mouse studies that have analyzed prepulse inhibition (PPI), a measure of sensorimotor gating that is abnormal in 22q11DS (57) as well as in several nonsyndromic psychiatric disorders including schizophrenia, posttraumatic stress disorder (reviewed in 58), Tourette's syndrome (58–60), and obsessive compulsive disorder (61). The PPI deficits observed in mice harboring large heterozygous deletions of the equivalent of the region commonly deleted in 22q11DS have been shown to be caused in part by haploinsufficiency of *Tbx1* (55,62). However, our own study failed to show sensorimotor gating deficits in mice heterozygous for loss of *Tbx1* (63).

We tested whether *TBX1* variation was associated with psychotic and affective disorders relevant to 22q11DS in a large cohort of white patients. Because psychiatric manifestations seen in 22q11 deletion syndrome include psychotic and affective diagnoses, we included in our analysis a total of 446 samples from patients diagnosed with schizophrenia, schizoaffective disorder, bipolar disorder, major depression, or psychotic and mood disorder not otherwise specified (NOS). To capture the largest amount of haplotypes possible, we determined the haplotype block structure of the *TBX1* locus and selected tag SNPs. To detect association of variants that directly influence *TBX1* expression levels, we included about 20 kb of the 5′ untranslated region. This interval contains several conserved elements showing high interspecies conservation, including a known functional enhancer (64).

All single-SNP as well as haplotype analyses failed to detect an association, leading us to conclude that *TBX1* is not likely to play a major role in the genetic etiology of nonsyndromic psychotic and affective disorders.

MATERIALS AND METHODS

Study Subjects

The study sample comprised 446 white patients residing in the USA (mean age = 36.2 years; 36.2% females). Diagnostic categories included schizophrenia (n = 226), schizoaffective disorder (n = 67), bipolar disorder (n = 82), major depression (n = 29), psychotic disorder NOS (20), and affective disorder NOS (22). The control group consisted of 436 white individuals (mean age = 38.73 years, 52.06% females).

Case patients were recruited from the inpatient and outpatient clinical services of the Zucker Hillside Hospital, a division of the North Shore–Long Island Jewish Health System (NSLIJHS). Inclusion criteria for screening for this study included a clinical diagnosis of a psychotic disorder, no active substance abuse, and ability to provide informed consent. After written informed consent was obtained, each subject was assessed with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID, version 2.0, 8/98), administered by trained raters. Standardized diagnostic assessments were supplemented with clinical information obtained by review of medical records and interviews with family informants when possible, and all diagnostic information was compiled into a narrative case summary. Information on the onset and course of axis I illness, presence of axis II pathology, presence of axis III diagnoses, and a brief description of the subject's psychosocial and occupational functioning during the course of illness was presented to a consensus diagnostic committee consisting of a minimum of three senior faculty with DSM-IV diagnostic experience, as well as other faculty and trainees with SCID experience. All available information was used to arrive at a consensus DSM-IV diagnosis.

Healthy controls were identified and recruited by the Zucker Hillside Hospital Healthy Control Project. Potential participants were recruited via local newspaper advertisements, flyers, and community Internet resources and underwent initial telephone screening to assess eligibility criteria. Subjects meeting eligibility criteria were administered the SCID-NP to rule out the presence of an axis I psychiatric disorder. A urine toxicology screen was conducted to rule out the use of any

drugs, and a family history questionnaire was conducted to rule out the presence of familial psychiatric disorders. Exclusion criteria included current or past: axis I psychiatric disorder, psychotropic drug treatment, substance abuse, first-degree family member with an axis I psychiatric disorder, or inability to provide written informed consent. A subset of the control cohort was collected through the Massachusetts General Hospital Clinical Research Program in conjunction with the Harvard-Partners Center for Genetics and Genomics in Boston, MA, USA. This subset comprised disease-free subjects older than 18 years. For the purposes of the study, "disease" was defined as current or past diagnoses made by a medical care provider that required medication or other forms of treatment/therapy. With the exception of orthopedic procedures, appendectomy, or trauma–related conditions, surgery was considered an exclusionary criterion. Subjects who took prescription medications or regularly used over-the-counter medications were also excluded. Subjects completed a structured family and medical questionnaire that detailed current and past psychiatric illness and pharmacological or psychotherapeutic psychiatric treatment. All responses to the self-report form were confirmed by clinical interview by physicians. Physical exams were completed at the study visit. This study was approved by the Institutional Review Board at NSLIJHS and Partners Healthcare.

The set of Caucasian control DNAs used to develop tag SNPs was obtained from Coriell (Camden, NJ, USA).

Tag SNP Selection

Forty-four SNPs were validated in a small set of normal controls. All SNPs that were monomorphic or had high failure rates were excluded from further analyses. Nineteen SNPs with a minor allele frequency of >5% across the *TBX1* locus were genotyped in a set of 94 Caucasian controls (Coriell). All but one SNP were derived from the dbSNP database. SNP (v14186) was derived through our own SNP discovery efforts and has not yet been assigned a dbSNP ID. All SNPs that were in Hardy-Weinberg equilibrium were then searched for contiguous subsets with reduced haplotype diversity. Together with custom-designed scripts, we used the programs PMPLUS (66) and EHPLUS (67), the latter of which employs the expectation-maximization algorithm to infer haplotype frequencies in the population. By sliding expanding and contracting windows along the region, we identified blocks of markers for which a large percentage of data were seen to be represented by just a few haplotypes. A reduced set of markers to tag the blocks was selected in such a way as to preserve the observed diversity among the major haplotypes (freq > 0.05). In situations for which more than one set of choices were available, we considered two additional factors: preference was given to markers with lower failure rates; marker subsets were chosen such that the greatest percentage of data

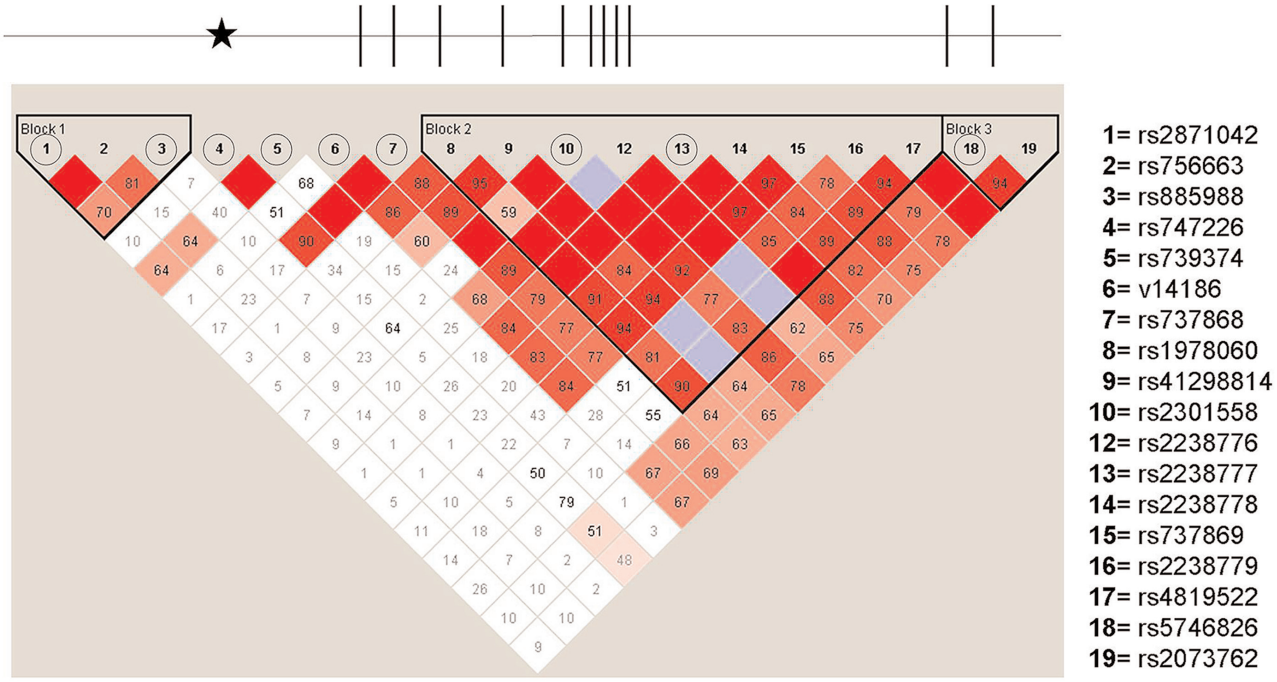

Figure 1. Linkage disequilibrium (LD) at the TBX1 locus and location of SNPs. The genomic organization of the TBX1 gene is shown above. Exons 1-9A, 9B, and 10 are symbolized by vertical bars. The star represents a known enhancer (64). Below, the underlying LD structure is shown. LD strength is symbolized by shades of red (the darker the higher). Solid black lines show three haplotype blocks. SNP identification numbers are listed on the right. SNPs selected for genotyping cases and controls are circled.

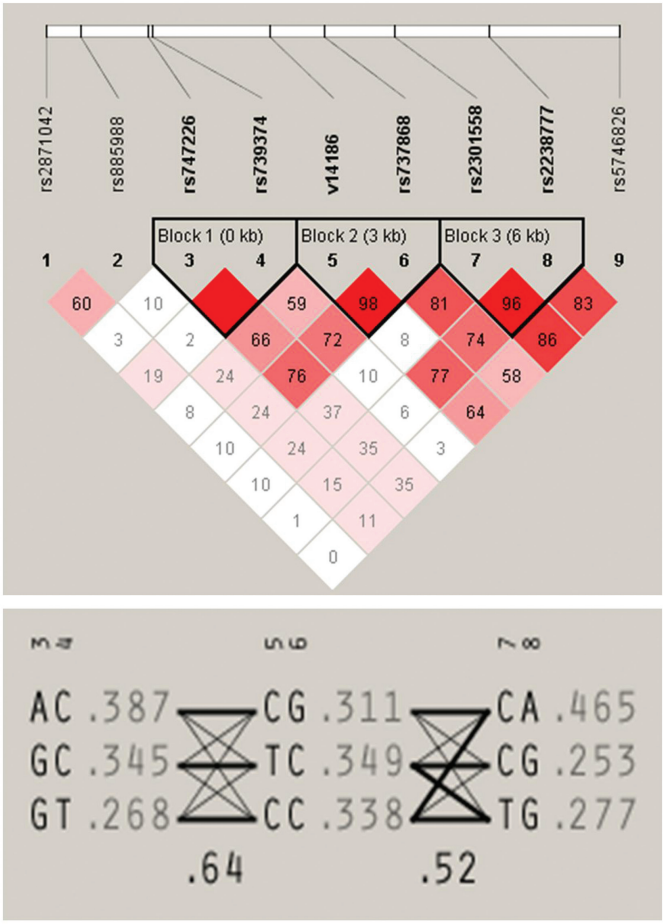

Figure 2. Linkage disequilibrium (LD) in the association sample. Pairwise LD between the nine SNPs genotyped in the association sample is shown in Table 1. Haplotypes as well as their frequencies are listed in Table 2.

could be pooled into the major haplotypes as defined by the tags.

SNP Genotyping

Multiplex PCR was carried out to generate short PCR products (>100 bp) containing one SNP or insertion deletion. Briefly, 2.5 ng genomic DNA was amplified in a 5 µL reaction containing 1 x HotStar Taq PCR buffer (Qiagen, Valencia, CA, USA), 2.5 mM $MgCl_2$, 200 µM each dNTP, 50 nM each PCR primer, and 0.1 U HotStar Taq (Qiagen). The reaction was incubated at 95 °C for 15 min followed by 45 cycles of 95 °C for 20 s, 56 °C for 30 s, 72 °C for 1 min, followed by 3 min at 72 °C. Excess dNTPs were then removed from the reaction by incubation with 0.3 U shrimp alkaline phosphatase (USB) at 37 °C for 20 min followed by 5 min at 85 °C to deactivate the enzyme. Single primer extension over the SNP or insertion/deletion was carried out in a final concentration of 600 nM each extension primer, 50 µM d/ddNTP and 0.126 U Thermosequenase (Solis Biodyne, Tartu, Estonia) and incubated at 94 °C for 2 min followed by 45 cycles of 94 °C for 5 s, 52 °C for 5 s, and 72 °C for 5 s. The reaction was then desalted by addition of a cation exchange resin followed by mixing and centrifugation to settle the contents of the tube. The extension product was then spotted onto a 384-well spectroCHIP before being flown in the MALDI-TOF mass spectrometer.

Statistical Analyses

Haplotype block structures for the nine tag SNPs were determined in the association study control sample using Haploview version 3.32 (68). Haplotype blocks were defined in accordance with the Gabriel method (69) with a minimum criterion for strong pairwise linkage disequilibrium (D′) between markers set to .90 and default settings for all other criteria. Using this method, three haplotype blocks were identified (Figure 1). Allelic and haplotypic χ^2 tests were conducted to test for association with any major affective or psychotic disorder (all affecteds), schizophrenia or schizoaffective disorder (schizophrenia and schizoaffective), schizophrenia only (schizophrenia), and affective disorder only (bipolar disorder and major depressive disorder). Allelic and haplotypic χ^2 tests were conducted to test for association using the same subgroups.

RESULTS

Because this study was initiated prior to completion of the Phase I HapMap, preliminary work was conducted to identify a set of SNPs with comprehensive, yet nonredundant coverage of the *TBX1* locus. First, 44 SNPs chosen from dbSNP and our own SNP discovery efforts as well as published studies (65) were validated in a small set of normal control samples. Of these, 19 SNPs with a minor allele frequency of >5% across the *TBX1* locus were genotyped in a set of 94 Caucasian controls. One SNP (rs2238775) was not in Hardy-Weinberg equilibrium and was excluded from further analysis. We reanalyzed our data with Haploview (68) because tag SNPs were generated before this now standard software was available. One tag SNP differed between our selection and those selected by Haploview with default settings. However, with a minor change in block definition, the tags selected by Haploview were identical to our initial selection. To define our blocks, we started with those defined by the Gabriel method (69) with the minimum fraction of strong linkage disequilibrium in in-

Table 1. Allele frequencies

SNP ID	Alleles (major/minor)	All affecteds: Allele	All affecteds: Frequency cases	All affecteds: Frequency controls	All affecteds: χ^2	All affecteds: *P*	Schizophrenia + schizoaffective: Allele	Schizophrenia + schizoaffective: Frequency cases	Schizophrenia + schizoaffective: Frequency controls	Schizophrenia + schizoaffective: χ^2	Schizophrenia + schizoaffective: *P*
			n = 446	n = 436				n = 293	n = 436		
rs2871042	A/G	A	0.53	0.53	0.002	0.963	A	0.54	0.53	0.039	0.843
rs885988	A/G	G	0.48	0.48	0.002	0.968	G	0.49	0.48	0.032	0.859
rs747226	G/A	G	0.62	0.60	1.025	0.311	G	0.63	0.60	0.919	0.338
rs739374	C/T	T	0.28	0.26	1.658	0.198	T	0.28	0.26	0.955	0.328
v14186	C/T	T	0.37	0.33	2.421	0.120	T	0.37	0.33	1.950	0.163
rs737868	C/G	C	0.71	0.67	2.682	0.102	C	0.72	0.67	3.546	0.060
rs2301558	C/T	C	0.72	0.71	0.170	0.680	C	0.71	0.71	0.002	0.961
rs2238777	A/G	G	0.54	0.52	1.165	0.280	G	0.55	0.52	1.401	0.237
rs5746826	G/T	T	0.54	0.51	1.238	0.266	T	0.55	0.51	1.768	0.184

SNP ID	Alleles (major/minor)	Schizophrenia: Allele	Schizophrenia: Frequency cases	Schizophrenia: Frequency controls	Schizophrenia: χ^2	Schizophrenia: *P*	Affective disorder (BP + MDD): Allele	Affective disorder (BP + MDD): Frequency cases	Affective disorder (BP + MDD): Frequency controls	Affective disorder (BP + MDD): χ^2	Affective disorder (BP + MDD): *P*
			n = 226	n = 436				n = 111	n = 436		
rs2871042	A/G	G	0.48	0.47	0.114	0.735	A	0.54	0.53	0.033	0.856
rs885988	A/G	G	0.49	0.48	0.125	0.723	A	0.52	0.48	0.001	0.971
rs747226	G/A	G	0.62	0.60	0.510	0.475	G	0.62	0.60	0.191	0.662
rs739374	C/T	T	0.29	0.26	1.694	0.193	T	0.31	0.26	2.490	0.115
v14186	C/T	T	0.36	0.33	1.080	0.299	T	0.34	0.33	0.080	0.777
rs737868	C/G	C	0.76	0.67	2.561	0.110	C	0.68	0.67	0.074	0.785
rs2301558	C/T	T	0.29	0.29	0.046	0.830	C	0.76	0.71	1.676	0.195
rs2238777	A/G	G	0.55	0.52	1.379	0.240	G	0.53	0.52	0.198	0.657
rs5746826	G/T	T	0.55	0.51	1.676	0.196	G	0.49	0.51	0.005	0.943

formative comparisons set to 0.8 and a minimum haplotype frequency of 5%, and simply extended them to include those flanking SNPs whose inclusion kept the sum of haplotypes captured above 85%. With these methods, we were able to eliminate the need to genotype 10 SNPs in high linkage disequilibrium with our tags, resulting in a final set of nine SNPs genotyped in our set of 446 samples of patients diagnosed with either schizophrenia, schizoaffective disorder, bipolar disorder or major depression, or psychotic and mood disorder NOS.

Figure 1 shows the *TBX1* genomic structure as well as the location of SNPs in relation to the underlying block structure. Figure 2 shows the pattern of linkage disequilibrium across the nine SNPs in the association sample. Three pairs of SNPs remained in very high linkage disequilibrium and formed haplotypes according to the modified Gabriel criteria described above. None of the nine individual SNPs showed a positive association in the "all affecteds" category (all nominal *P* values > 0.05), nor did any of the three haplotypes (all nominal *P* values > 0.05). Similarly, analysis of the individual subgroups of patients compared with healthy controls, (schizophrenia/schizoaffective, n = 293; schizophrenia only, n = 226; affective disorder n = 111) remained entirely negative (Table 1) as did haplotype analysis of the same patient categories (Table 2).

DISCUSSION

22q11DS is one of the strongest genetic risk factors for the development of schizophrenia, and the region deleted in patients with 22q11DS is assumed to contain one or several genes that are also involved in the genetic etiology of nonsyndromic schizophrenia. Based on the hypothesis that disturbed brain development contributes to schizophrenia and the fact that haploinsufficiency of *TBX1* causes the main developmental anomalies of 22q11DS, we analyzed a possible contribution of *TBX1* variation to the genetic etiology of schizophrenia and other nonsyndromic psychiatric disorders seen in patients with 22q11DS.

We genotyped a set of tag SNPs across the *TBX1* gene in a set of 446 white patients and 436 ethnically matched controls. Allele and haplotype frequencies did not differ significantly between all affected patients and controls, and no significant differences were observed between cases and controls in the diagnostic subcategories.

Our negative findings can be interpreted several ways. First, *TBX1* may not

Table 2. Haplotype frequencies

	All affecteds					Schizophrenia + schizoaffective				
Haplotype	Frequency cases	Frequency controls	χ^2	*P*	Within-block *P*	Frequency cases	Frequency controls	χ^2	*P*	Within-block *P*
Block 1 (rs747226+739374)										
AC	0.375	0.399	1.086	0.297	0.385	0.37	0.40	0.946	0.331	0.530
GC	0.343	0.346	0.015	0.902		0.35	0.35	0.010	0.919	
GT	0.282	0.255	1.635	0.201		0.28	0.25	0.935	0.334	
Block 2 (v14186+rs737864)										
TC	0.366	0.332	2.100	0.147	0.213	0.37	0.33	1.805	0.179	0.176
CC	0.338	0.338	0.001	0.982		0.35	0.34	0.119	0.731	
CG	0.293	0.329	2.488	0.115		0.28	0.33	3.262	0.071	
Block 3 (rs2301558+rs2238777)										
CA	0.456	0.475	0.609	0.435	0.543	0.44	0.47	0.869	0.351	0.530
TG	0.278	0.276	0.011	0.915		0.28	0.28	0.127	0.722	
CG	0.264	0.241	1.273	0.259		0.26	0.24	1.018	0.313	
	Schizophrenia					Affective disorder				
Haplotype	Frequency cases	Frequency controls	χ^2	*P*	Within-block *P*	Frequency cases	Frequency controls	χ^2	*P*	Within-block *P*
Block 1 (rs747226+739374)										
AC	0.379	0.400	0.537	0.464	0.411	0.38	0.40	0.189	0.664	0.275
GC	0.333	0.346	0.232	0.630		0.31	0.35	1.018	0.313	
GT	0.289	0.255	1.760	0.185		0.31	0.25	2.444	0.118	
Block 2 (v14186+rs737864)										
TC	0.362	0.333	1.033	0.310	0.301	0.34	0.34	0.000	0.9993	0.975
CC	0.350	0.338	0.197	0.657		0.34	0.33	0.014	0.9065	
CG	0.288	0.329	2.221	0.136		0.32	0.33	0.063	0.8022	
Block 3 (rs2301558+rs2238777)										
CA	0.446	0.474	0.954	0.329	0.571	0.47	0.47	0.053	0.8175	0.385
TG	0.291	0.276	0.338	0.561		0.25	0.28	0.733	0.3921	
CG	0.261	0.241	0.645	0.422		0.29	0.24	1.846	0.1743	

play a role in the genetic etiology of the nonsyndromic versions of the psychiatric disorders included in this study. This finding would be concordant with results of our own study showing that mice with heterozygous loss of *Tbx1* do not show the same sensorimotor gating deficits observed in mice carrying large deletions affecting *Tbx1* and 26 other genes (63). The lack of association would also be supported by data published by Hiroi et al who found that although the behavioral anomalies of mice transgenic for a segment containing four genes including *TBX1* could be ameliorated by antipsychotic drugs, this effect was not observed in mice heterozygous for *TBX1* loss (70). However, our findings contrast with those of a study by Paylor et al (55), who showed that prepulse inhibition (PPI) defects seen in a mouse model of 22q11DS are caused by haploinsufficiency of two genes in the deleted region, *Tbx1* and *Gnb1l*. PPI is a measurable endophenotype of several psychiatric disorders including schizophrenia and can be measured in humans as well as mice. Of note, the mouse studies by Long et al (63) and Paylor et al (55) were both carried out in mice with a mixed genetic background and are difficult to compare because strain-dependent penetrance of the sensorimotor gating deficit cannot be excluded. Alternatively, the lack of *TBX1* association in our sample may be a false-negative result. Of note, associations of two genes, *DTNBP1* and *COMT*, have been shown in the same cohort (71,72). Statistical power is a common concern when considering negative results in genetic association studies of complex disease. It is clear that psychiatric illnesses are polygenic, with any single genetic locus likely to contribute only a small proportion of the risk variance (73). Consequently, association studies should be adequately powered to detect effect sizes (odds ratio, OR) of 1.5 or less. Given the sample sizes of cases and controls in the present study, we would have had approximately 80% power to detect a similar effect for *TBX1*. Furthermore, all *TBX1* SNPs and haplotypes examined in the present study had a robust minor allele frequency of 25% or greater. The present study would have had power of 70%–80% to detect effects as low as OR = 1.3 for all such variants, and had nearly 100% power to detect ORs > 1.5. Thus, our results are unlikely to be false negatives, and even moderate effects for *TBX1* on major psychiatric illness can be excluded. However, it is still possible

that very small effects (1 < OR < 1.3) were not detected.

In summary, it is unlikely that *TBX1* plays a major role in the genetic etiology of nonsyndromic schizophrenia and other psychiatric disorders observed in patients with 22q11DS. However, it is important to point out that schizophrenia associated with 22q11DS may represent a genetic subclass that is very similar but genetically distinct from the nonsyndromic form (13,74,75). In this case *TBX1* may still contribute to the risk of developing psychiatric disease in patients with 22q11DS.

ACKNOWLEDGMENTS

This project was supported by grants HD034980-09 to RK, K23MH001760 to AKM, K01 MH65580 to TL, P30 MH60575 to JK, and a General Clinical Research Center (M01 RR18535). We thank the HPCGG Genotyping core for their fast and excellent service and Dr. Bernice Morrow for providing services to generate lymphoblastoid cell lines and DNA.

REFERENCES

1. Ryan AK, Goodship JA, Wilson DI, et al. (1997) Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *J. Med. Genet.* 34:798–804.
2. Goldberg R, Motzkin B, Marion R, Scambler PJ, Shprintzen RJ. (1993) Velo-cardio-facial syndrome: a review of 120 patients. *Am. J. Med. Genet.* 45:313–9.
3. Lindsay EA, Goldberg R, Jurecic V, et al. (1995) Velo-cardio-facial syndrome: frequency and extent of 22q11 deletions. *Am. J. Med. Genet.* 57:514–22.
4. Shaikh TH, Kurahashi H, Saitta SC, et al. (2000) Chromosome 22-specific low copy repeats and the 22q11.2 deletion syndrome: genomic organization and deletion endpoint analysis. *Hum. Mol. Genet.* 9:489–501.
5. Carlson C, Sirotkin H, Pandita R, et al. (1997) Molecular definition of 22q11 deletions in 151 velo-cardio-facial syndrome patients. *Am. J. Hum. Genet.* 61:620–9.
6. Baker KD, Skuse DH. (2005) Adolescents and young adults with 22q11 deletion syndrome: psychopathology in an at-risk group. *Br. J. Psychiatry* 186:115–20.
7. Gothelf D, Gruber R, Presburger G, et al. (2003) Methylphenidate treatment for attention-deficit/hyperactivity disorder in children and adolescents with velocardiofacial syndrome: an open-label study. *J. Clin. Psychiatry* 64:1163–69.
8. Gothelf D, Presburger G, Zohar AH, et al. (2004) Obsessive-compulsive disorder in patients with velocardiofacial (22q11 deletion) syndrome. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 126:99–105.
9. Papolos DF, Faedda GL, Veit S, et al. (1996) Bipolar spectrum disorders in patients diagnosed with velo-cardio-facial syndrome: does a hemizygous deletion of chromosome 22q11 result in bipolar affective disorder? *Am. J. Psychiatry* 153:1541–7.
10. Murphy KC. (2005) Annotation: velo-cardio-facial syndrome. *J. Child Psychol. Psychiatry* 46:563–71.
11. Fine SE, Weissman A, Gerdes M, et al. (2005) Autism spectrum disorders and symptoms in children with molecularly confirmed 22q11.2 deletion syndrome. *J. Autism Dev. Disord.* 35:461–70.
12. Bassett AS, Chow EW, AbdelMalik P, Gheorghiu M, Husted J, Weksberg R. (2003) The schizophrenia phenotype in 22q11 deletion syndrome. *Am. J. Psychiatry* 160:1580–6.
13. Murphy KC, Jones LA, Owen MJ. (1999) High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Arch. Gen. Psychiatry* 56:940–5.
14. Shprintzen RJ, Goldberg R, Golding-Kushner KJ, Marion RW. (1992) Late-onset psychosis in the velo-cardio-facial syndrome. *Am. J. Med. Genet.* 42:141-142.
15. Murphy KC. (2002) Schizophrenia and velo-cardio-facial syndrome. *Lancet* 359:426–30.
16. Mukai J, Liu H, Burt RA, et al. (2004) Evidence that the gene encoding ZDHHC8 contributes to the risk of schizophrenia. *Nat. Genet.* 36:725–31.
17. Sinibaldi L, De Luca A, Bellacchio E, et al. (2004) Mutations of the Nogo-66 receptor (RTN4R) gene in schizophrenia. *Hum. Mutat.* 24:534–5.
18. Sanders AR, Rusu I, Duan J, et al. (2005) Haplotypic association spanning the 22q11.21 genes COMT and ARVCF with schizophrenia. *Mol. Psychiatry* 10:353–65.
19. Sun ZY, Wei J, Xie L, et al. (2004) The CLDN5 locus may be involved in the vulnerability to schizophrenia. *Eur. Psychiatry* 19:354–7.
20. Ye L, Sun Z, Xie L, et al. (2005) Further study of a genetic association between the CLDN5 locus and schizophrenia. *Schizophr. Res.* 75:139–41.
21. Wang H, Duan S, Du J, et al. (2006) Transmission disequilibrium test provides evidence of association between promoter polymorphisms in 22q11 gene DGCR14 and schizophrenia. *J. Neural Transm.* 113:1551–61.
22. De Luca A, Conti E, Grifone N, et al. (2003) Association study between CAG trinucleotide repeats in the PCQAP gene (PC2 glutamine/Q-rich-associated protein) and schizophrenia. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 116:32–5.
23. De Luca A, Pasini A, Amati F, et al. (2001) Association study of a promoter polymorphism of UFD1L gene with schizophrenia. *Am. J. Med. Genet.* 105:529–33.
24. Saito T, Guan F, Papolos DF, Rajouria N, Fann CS, Lachman HM. (2001) Polymorphism in SNAP29 gene promoter region associated with schizophrenia. *Mol. Psychiatry* 6:193–201.
25. Takase K, Ohtsuki T, Migita O, et al. (2001) Association of ZNF74 gene genotypes with age-at-onset of schizophrenia. *Schizophr. Res.* 52:161–5.
26. Finn CT FB, Kikinis Z, Shenton M, Schiripo T. Psychiatric manifestations of velocardiofacial syndrome. In: Morrow EM, Rosenbaum JF, Fava M, Biederman J, editors. *Frontiers in Biological Psychiatry in Childhood: Exploring the Relationship among Genes, Brain Development, and the Emergence of Psychopathology*. Arlington (VA): American Psychiatric Publishing, Inc. In press.
27. Owen MJ, Craddock N, O'Donovan MC. (2005) Schizophrenia: genes at last? *Trends Genet.* 21:518–25.
28. Harrison PJ, Weinberger DR. (2005) Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol. Psychiatry* 10:40–68.
29. Collier DA, Li T. (2003) The genetics of schizophrenia: glutamate not dopamine? *Eur. J. Pharmacol.* 480:177–84.
30. Efron ML. (1965) Familial hyperprolinemia: report of a second case, associated with congenital renal malformations, hereditary hematuria and mild mental retardation, with demonstration of an enzyme defect. *N. Engl. J. Med.* 272:1243–54.
31. Paterlini M, Zakharenko SS, Lai WS, et al. (2005) Transcriptional and behavioral interaction between 22q11.2 orthologs modulates schizophrenia-related phenotypes in mice. *Nat. Neurosci.* 8:1586–94.
32. Gogos JA, Santha M, Takacs Z, et al. (1999) The gene encoding proline dehydrogenase modulates sensorimotor gating in mice. *Nat. Genet.* 21:434–9.
33. Liu H, Heath SC, Sobin C, et al. (2002) Genetic variation at the 22q11 PRODH2/DGCR6 locus presents an unusual pattern and increases susceptibility to schizophrenia. *Proc. Natl. Acad. Sci. U. S. A.* 99:3717–22.
34. Li T, Ma X, Sham PC, et al. (2004) Evidence for association between novel polymorphisms in the PRODH gene and schizophrenia in a Chinese population. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 129:13–15.
35. Williams HJ, Williams N, Spurlock G, et al. (2003) Association between PRODH and schizophrenia is not confirmed. *Mol. Psychiatry* 8:644–5.
36. Williams HJ, Williams N, Spurlock G, et al. (2003) Detailed analysis of PRODH and PsPRODH reveals no association with schizophrenia. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 120:42–6.
37. Fan JB, Ma J, Zhang CS, et al. (2003) A family-based association study of T1945C polymorphism in the proline dehydrogenase gene and schizophrenia in the Chinese population. *Neurosci. Lett.* 338:252–4.
38. Axelrod J. (1966) Methylation reactions in the formation and metabolism of catecholamines and other biogenic amines. *Pharmacol. Rev.* 18:95–113.

39. Karayiorgou M, Gogos JA. (2004) The molecular genetics of the 22q11-associated schizophrenia. *Brain Res. Mol. Brain Res.* 132:95–104.
40. Liu H, Abecasis GR, Heath SC, et al. (2002) Genetic variation in the 22q11 locus and susceptibility to schizophrenia. *Proc. Natl. Acad. Sci. U. S. A.* 99:16859–64.
41. Faul T, Gawlik M, Bauer M, et al. (2005) ZDHHC8 as a candidate gene for schizophrenia: analysis of a putative functional intronic marker in case-control and family-based association studies. *BMC Psychiatry* 5:35.
42. Otani K, Ujike H, Tanaka Y, et al. (2005) The ZDHHC8 gene did not associate with bipolar disorder or schizophrenia. *Neurosci. Lett.* 390:166–70.
43. Saito S, Ikeda M, Iwata N, et al. (2005) No association was found between a functional SNP in ZDHHC8 and schizophrenia in a Japanese case-control population. *Neurosci. Lett.* 374:21–4.
44. Glaser B, Moskvina V, Kirov G, et al. (2006) Analysis of ProDH, COMT and ZDHHC8 risk variants does not support individual or interactive effects on schizophrenia susceptibility. *Schizophr. Res.* 87:21–7.
45. Glaser B, Schumacher J, Williams HJ, et al. (2005) No association between the putative functional ZDHHC8 single nucleotide polymorphism rs175174 and schizophrenia in large European samples. *Biol. Psychiatry* 58:78–80.
46. Rapoport JL, Addington AM, Frangou S, Psych MR. (2005) The neurodevelopmental model of schizophrenia: update 2005. *Mol. Psychiatry* 10:434–49.
47. Guy JD, Majorski LV, Wallace CJ, Guy MP. (1983) The incidence of minor physical anomalies in adult male schizophrenics. *Schizophr. Bull.* 9:571–82.
48. Waddington JL, Lane A, Scully P, et al. (1999) Early cerebro-craniofacial dysmorphogenesis in schizophrenia: a lifetime trajectory model from neurodevelopmental basis to 'neuroprogressive' process. *J. Psychiatr. Res.* 33:477–89.
49. Lindsay EA, Baldini A. (2001) Recovery from arterial growth delay reduces penetrance of cardiovascular defects in mice deleted for the DiGeorge syndrome region. *Hum. Mol. Genet.* 10:997–1002.
50. Arnold JS, Werling U, Braunstein EM, et al. (2006) Inactivation of Tbx1 in the pharyngeal endoderm results in 22q11DS malformations. *Development* 133:977–87.
51. LaMantia AS. (1999) Forebrain induction, retinoic acid, and vulnerability to schizophrenia: insights from molecular and genetic analysis in developing mice. *Biol. Psychiatry* 46:19–30.
52. Maynard TM, Sikich L, Lieberman JA, LaMantia AS. (2001) Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia. *Schizophr. Bull.* 27:457–76.
53. Maynard TM, Haskell GT, Peters AZ, Sikich L, Lieberman JA, LaMantia AS. (2003) A comprehensive analysis of 22q11 gene expression in the developing and adult brain. *Proc. Natl. Acad. Sci. U. S. A.* 100:14433–8.
54. Lindsay EA, Vitelli F, Su H, et al. (2001) Tbx1 haploinsufficieny in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature* 410:97–101.
55. Paylor R, Glaser B, Mupo A, et al. (2006) Tbx1 haploinsufficiency is linked to behavioral disorders in mice and humans: implications for 22q11 deletion syndrome. *Proc. Natl. Acad. Sci. U. S. A.* 103:7729–34.
56. Meechan DW, Maynard TM, Wu Y, Gopalakrishna D, Lieberman JA, LaMantia AS. (2006) Gene dosage in the developing and adult brain in a mouse model of 22q11 deletion syndrome. *Mol. Cell Neurosci.* 33:412–28.
57. Sobin C, Kiley-Brabeck K, Karayiorgou M. (2005) Lower prepulse inhibition in children with the 22q11 deletion syndrome. *Am. J. Psychiatry* 162:1090–9.
58. Braff DL, Geyer MA, Swerdlow NR. (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl.)* 156:234–58.
59. Castellanos FX, Fine EJ, Kaysen D, Marsh WL, Rapoport JL, Hallett M. (1996) Sensorimotor gating in boys with Tourette's syndrome and ADHD: preliminary results. *Biol. Psychiatry* 39:33–41.
60. Swerdlow NR, Karban B, Ploum Y, Sharp R, Geyer MA, Eastvold A. (2001) Tactile prepuff inhibition of startle in children with Tourette's syndrome: in search of an "fMRI-friendly" startle paradigm. *Biol. Psychiatry* 50:578–85.
61. Hoenig K, Hochrein A, Quednow BB, Maier W, Wagner M. (2005) Impaired prepulse inhibition of acoustic startle in obsessive-compulsive disorder. *Biol. Psychiatry* 57:1153–8.
62. Paylor R, McIlwain KL, McAninch R, et al. (2001) Mice deleted for the DiGeorge/velocardiofacial syndrome region show abnormal sensorimotor gating and learning and memory impairments. *Hum. Mol. Genet.* 10:2645–50.
63. Long JM, Laporte P, Merscher S, et al. (2006) Behavior of mice with mutations in the conserved region deleted in velocardiofacial/DiGeorge syndrome. *Neurogenetics* 7:247–57.
64. Yamagishi H, Maeda J, Hu T, et al. (2003) Tbx1 is regulated by tissue-specific forkhead proteins through a common Sonic hedgehog-responsive enhancer. *Genes Dev.* 17:269–81.
66. Zhao JH, Curtis D, Sham PC. (2000) Model-free analysis and permutation tests for allelic associations. *Hum. Hered.* 50:133–9.
67. Xie X OJ. (1993) Testing linkage disequilibrium between a disease gene and marker loci (abstract). *Am. J. Hum. Genet.* 53:1107.
68. Barrett JC, Fry B, Maller J, Daly MJ. (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263–5.
69. Gabriel SB, Schaffner SF, Nguyen H, et al. (2002) The structure of haplotype blocks in the human genome. *Science* 296:2225–9.
70. Hiroi N, Zhu H, Lee M, et al. (2005) A 200-kb region of human chromosome 22q11.2 confers antipsychotic-responsive behavioral abnormalities in mice. *Proc. Natl. Acad. Sci. U. S. A.* 102:19132–7.
71. Funke B, Malhotra AK, Finn CT, et al. (2005) COMT genetic variation confers risk for psychotic and affective disorders: a case control study. *Behav. Brain Funct.* 1:19.
72. Funke B, Finn CT, Plocik AM, et al. (2004) Association of the DTNBP1 locus with schizophrenia in a U.S. population. *Am. J. Hum. Genet.* 75:891–8.
73. Kendler KS. (2005) "A gene for...": the nature of gene action in psychiatric disorders. *Am. J. Psychiatry* 162:1243–52.
74. Bassett AS, Chow EW. (1999) 22q11 deletion syndrome: a genetic subtype of schizophrenia. *Biol. Psychiatry* 46:882–1.
75. Sporn A, Addington A, Reiss AL, et al. (2004) 22q11 deletion syndrome in childhood onset schizophrenia: an update. *Mol. Psychiatry* 9:225–6.