Association Between Schizophrenia and the Syntaxin 1A Gene

Albert H.C. Wong, Joseph Trakalo, Olga Likhodi, Muneeb Yusuf, Antonio Macedo, Maria-Helena Azevedo, Tim Klempan, Michele T. Pato, William G. Honer, Carlos N. Pato, Hubert H.M. Van Tol, and James L. Kennedy

Background: *Both microarray and candidate molecule studies have demonstrated that protein and mRNA expression of syntaxin and other genes involved in synaptic function are altered in the cerebral cortex of patients with schizophrenia.*

Methods: *Genetic association between polymorphic markers in the syntaxin 1A gene and schizophrenia was assessed in a matched case-control sample of 192 pairs, and in an independent sample of 238 nuclear families.*

Results: *In the family-based sample, a significant genetic association was found between schizophrenia and one of the four single nucleotide polymorphisms (SNPs) tested: an intron 7 SNP (transmission disequilibrium test [TDT]* $\chi^2 = 5.898$; df = 1; p = .015, *family-based association test [FBAT]* z = 2.280, p = .023*). When the results for the TDT and case-control analyses were combined, the association was stronger (*n = 430; $z_c = 2.859$; p = .004*). Haplotype analysis supported the association with several significant values that appear to be driven by the intron 7 SNP.*

Conclusions: *The results should be treated with caution until replicated, but this is the first report of a genetic association between syntaxin 1A and schizophrenia.*

Key Words: Schizophrenia, genetics, syntaxin 1A, synaptic protein, antipsychotics

Syntaxin is a protein enriched in presynaptic terminals (Bennett et al 1992), and together with synaptosome-associated protein 25 kDa (SNAP-25) and vesicle-associated membrane protein (VAMP), forms the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, which is vital for chemical neurotransmission (Sollner et al 1993). Within presynaptic terminals, syntaxin binds to a host of other neurotransmitter-associated proteins, including the gamma aminobutyric acid and norepinephrine (NET) transporters (Quick 2002; Sung et al 2003). Syntaxin-NET binding modulates both the intrinsic activity of the transporter and transporter trafficking to the plasma membrane surface (Sung et al 2003). In one of the few studies of physiologic function, syntaxin was transiently downregulated in rat nucleus accumbens following administration of lipopolysaccharide (Barr et al 2003).

Independent postmortem studies in schizophrenia provide evidence of increased syntaxin immunoreactivity in the cingulate cortex of schizophrenia brains when compared with controls (Gabriel et al 1997; Honer et al 1997). Syntaxin immunoreactivity was unchanged in schizophrenia in other brain regions including frontal, temporal, and parietal cortices (Gabriel et al 1997), as well as cerebellum (Mukaetova-Ladinska et al 2002). A study of the temporal cortex in schizophrenia patients showed higher syntaxin mRNA levels in samples of younger patients when compared with controls, and a negative correlation of mRNA levels with age in patient samples but not in controls (Sokolov et al 2000). These studies suggest a possible role for syntaxin in the pathogenesis of schizophrenia through disruption of the SNARE complex, or interference with neurotransmitter trafficking.

We hypothesized that one or more polymorphisms of the syntaxin 1A gene (STX1A) would demonstrate a genetic association with schizophrenia, and examined this hypothesis across four polymorphisms in both case-control and family-based samples.

Methods and Materials

Patient Recruitment and Sample Collection

Subjects for this study were recruited with fully informed written consent, and in accordance with University of Toronto and Medical Research Council of Canada guidelines for the ethical treatment of human subjects. Study approval for the recruitment of Portuguese subjects was given by the University of Coimbra Internal Review Board (IRB), and in the United States by the IRB of the State University of New York, Buffalo. A total of 238 nuclear families consisting of probands with schizophrenia and at least one first-degree relative were collected from both mainland Portugal (Coimbra) and the Azores islands (124 families), and from hospitals in Toronto, Ontario (114 families). In addition, 192 case-control pairs were recruited from the Toronto area. All patients had an independent clinical DSM-III-R/DSM-IV diagnosis of schizophrenia from their referring psychiatrist (American Psychiatric Association 1994). In patients from Portugal and the Azores, the diagnosis was confirmed with the Diagnostic Interview for Genetic Studies (Nurnberger et al 1994). A Structured Clinical Interview for Diagnosis was administered by trained research assistants to each proband from the Toronto region to confirm a DSM-III-R diagnosis of schizophrenia. In both samples, a consensus-based procedure provided the ultimate decision for the diagnostic classification of the patients. Controls were screened using the Family Interview for Genetic Studies (Maxwell 1992) and excluded if there was any personal or family history of mental illness or alcohol/substance abuse. Patients and control subjects were matched for age, gender, and self-reported

From the Centre for Addiction and Mental Health (AHCW, JT, OL, TK, HHMVT, JLK), Department of Psychiatry (AHCW, HHMVT, JLK), Department of Pharmacology (AHCW, MY, HHMVT), and Institute of Medical Science (AHCW, TK, HHMVT, JLK) Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada; Department of Psychiatry (AM, M-HA), University of Coimbra, Portugal; Department of Psychiatry and Behavioral Sciences (MTP, CNP), Upstate Medical University, State University of New York, Syracuse, New York; Research Division (MTP, CNP), Veterans Affairs Medical Center, Washington D.C.; Centre for Complex Disorders (WGH), University of British Columbia, Vancouver, British Columbia, Canada

Address reprint requests to Dr. Albert H.C. Wong, Room 711, Centre for Addiction and Mental Health, 250 College Street, Toronto, ON, Canada, M5T 1R8.

Received May 6, 2003; revised February 18, 2004; accepted March 5, 2004.

0006-3223/04/$30.00
doi:10.1016/j.biopsych.2004.03.008

ethnicity (Rosenberg et al 2002) to reduce the potential stratification that might result from ethnically heterogeneous case and control groups. Venous blood was obtained from subjects using standard venipuncture techniques. DNA was extracted using a nonenzymatic high-salt procedure (Lahiri and Nurnberger 1991).

Genotyping

The syntaxin 1A gene (located at 7q11.2; Nakayama et al 1998) was initially genotyped at two restriction fragment length polymorphisms (RFLPs); one each in exon 3 and intron 7 (Tsunoda et al 2001). After reviewing the preliminary data, we genotyped two more markers in the family-based samples, a single nucleotide polymorphism (SNP) in intron 3 (refSNP ID: rs3793243; NCBI dbSNP# ss4979617) and another SNP in the 3′-untranslated region (3′UTR) (rs1569061; ss2413383).

The exon 3 polymorphism, a silent T/C substitution (D68D), was amplified with the following polymerase chain reaction (PCR) primers: 5′- CTACTCTGGGCCATCTCTG (forward) and 5′- CAGAGGTCCCGTGAGGCCTC (reverse). The PCR cycling conditions were: 95°C × 5 min; followed by 35 cycles of 95°C × 30sec, 62°C × 30sec, 72°C × 30sec. The exon 3 PCR product was mixed with .5 μl Hpy99I, 3 μl Buffer 4, .3 μL 100 × BSA (all from NEB) 1.2 μl H_2O, and digested at 37°C×1 hour. Electrophoresis on a 2.5% agarose gel at 100 V for 60 min allowed visualization of a 263 base pair (bp) uncut band or 174 bp/89 bp cut bands. The intron 7 RFLP is also a T/C substitution, 52 bp downstream from the 3′ end of exon 7. The primer sequences were: 5′- CAATGCTGCTGCTGAACTC (forward) and 5′- CGCTGACATTTATGTGACC (reverse) and the cycling conditions were: 95°C×5 min; followed by 35 cycles of 95°C×30sec, 60°C×30sec, 72°C×30sec. The intron 7 PCR product was mixed with .2μl HpyCH4IV, 3 μl Buffer 1 (both from New England Biolabs, Inc., Beverly, Massachusetts), 1.8 μl H_2O, and digested at 37°C×1 hour. Electrophoresis on a 2.5% agarose gel at 100V for 60 min allowed visualization of a 312-bp uncut band or 186 bp/126 bp cut bands. The 3′UTR SNP was genotyped with fluorescent TaqMan probes as part of commercial Assays-on-Demand SNP Genotyping assays on the ABI PRISM 7000 sequence detection system (assay #C 431715 10, Applied Biosystems Inc., Foster City, California). The intron 3 SNP was likewise genotyped with a Taqman assay developed under Applied Biosystems' Assays-by-Design service.

Genotypes were independently read by two authors (O.L. & J.T.), who were blind to the identity of the individual and the relationship of the individual to other samples. Any ambiguous genotypes were repeated and ultimately discarded if they remained unclear. Mendelian errors were identified and families in which false paternity was suspected were discarded.

Population Comparisons and Population Genetics

We compared the family-based sample groups for mean age, age of onset of schizophrenia, and gender distribution using chi-square and *t*-test procedures for categorical and continuous variables respectively. The ethnic composition of the Toronto families was: 75.9% European Caucasian, 4.5% East Indian Caucasian, 3.6% Asian, .9% Hispanic, and 15.1% Mixed (includes some combination of European, African, Native North American, and West Indian). Because the Toronto and Portuguese samples represent ethnically mixed and homogeneous populations respectively, it was necessary to determine if the populations can be combined in a genetic analysis. Population level differences in genetic diversity at the STX1A locus were assessed using multiple tests to determine with reasonable certainty that the two populations did not have significant genetic differences at this locus. Four populations were defined according to generation and study group. Because human populations contain overlapping generations, the F1 generation in a given family was defined by the proband and included all siblings. The parental generation, including aunts and uncles, was defined as the F0 generation. F2 and subsequent generations were not included in the analysis as these individuals were rare. Hence, four populations were described: Toronto F0 (TS_{F0}), Toronto F1 (TS_{F1}), Portuguese F0 (PS_{F0}), and Portuguese F1 (PS_{F1}). We assessed for significant differences in allele frequencies across the two populations by generation using a chi-square test of the null hypothesis that there is no difference between the frequencies in the two populations. In addition, we used the haplotypes generated from all four polymorphisms to assess for genetic structure. Haplotype frequencies were estimated in Arlequin v.2.0 using the expectation-maximization (EM) algorithm (Schneider et al 2000). Only individuals with complete haplotypes were used in the tests for genetic structure. Genetic structure was tested on three levels. The fixation index, F_{ST}, was measured from pairwise distances calculated among the four populations. In addition, a locus-by-locus analysis of molecular variance (AMOVA) was computed to assess for differences on a polymorphism level. Finally, an exact test of population differentiation was calculated using haplotypes. All population-level comparisons were performed using Arlequin v.2.0 (Schneider et al 2000). A likelihood ratio test of linkage disequilibrium (LD) between the four polymorphisms for genotype data with gametic phase unknown (Slatkin and Excoffier 1996) and tests of concordance with Hardy-Weinberg equilibrium (HWE) were also performed in Arlequin v.2.0. Because gametic phase of the haplotypes was unknown, it was not possible to calculate LD for haplotypes.

Genetic Statistical Analysis

The association between schizophrenia and the STX1A polymorphisms was assessed with the transmission disequilibrium test (TDT; Spielman et al 1993), using the TDT-sTDT Program v1.1 (Spielman and Ewens 1998). The family-based association test (FBAT) was also used to include additional families in the analysis that had one parent and unaffected sibs or other family constellations (Laird et al 2000). Haplotypes were analyzed with the TRANSMIT software, which generalizes the transmission disequilibrium test to the analysis of adjacent loci (Clayton and Jones 1999). The case-control samples were assessed for association between schizophrenia and the two RFLPs in exon 3 and intron 7, using the basic chi-square test with both genotypes and individual alleles. To examine the overall effect of the syntaxin 1A exon 3 and intron 7 polymorphisms in the two independent samples of case controls and nuclear families, we converted the two chi-square values to corresponding z scores using the formula $(\chi^2 - n)/\sqrt{(2n)}$, where n is the degrees of freedom for the χ^2 value. We then obtained the combined overall z score using the inverse normal method by adding the two z scores and dividing the sum by the square root of 2 (Hedges et al 1985).

Results

Population Comparisons

The mean age of the Toronto and Portuguese subjects was 34.3 and 33.6 years respectively, and mean age of onset (when available) was 19.6 and 22.1 years respectively. For the combined sample, the mean age was 33.9 years and the mean age of onset

Table 1. Allele and Genotype Frequencies

	Allele Frequencies								Genotype Frequencies											
	Exon 3		Intron 3		Intron 7		3′ UTR		Exon 3			Intron 3			Intron 7			3′ UTR		
	A1	A2	A1	A2	A1	A2	A1	A2	11	12	22	11	12	22	11	12	22	11	12	22
PS_{F0}	.424	.576	.946	.054	.409	.591	.510	.490	.203	.441	.356	.893	.107	.00	.174	.471	.355	.277	.465	.257
TS_{F0}	.535	.465	.911	.089	.417	.583	.580	.420	.220	.630	.150	.829	.164	.007	.181	.472	.346	.333	.492	.174
F0	$\chi^2 = 3.587$, 1 *df* $p = .058$		$\chi^2 = 1.838$, 1 *df* $p = .175$		$\chi^2 = .009$, 1 *df* $p = .924$		$\chi^2 = 1.970$, 1 *df* $p = .161$		.215	.570	.215	.857	.139	.004	.177	.472	.351	.309	.481	.210
PS_{F1}	.469	.531	.941	.059	.413	.587	.525	.475	.160	.617	.222	.882	.118	.00	.145	.536	.319	.285	.481	.234
TS_{F1}	.522	.478	.888	.112	.354	.646	.525	.475	.225	.594	.181	.782	.211	.007	.143	.421	.436	.290	.471	.239
F1	$chi^2 = .929$, 1 *df* $p = .335$		$\chi^2 = 5.095$, 1 *df* $p = .024$		$\chi^2 = 1.836$, 1 *df* $p = .175$		$\chi^2 = .000$, 1 *df* $p = .999$		.201	.613	.196	.835	.161	.003	.144	.478	.378	.287	.476	.236

F0, parental generation (includes aunts and uncles); F1, proband (includes all siblings); PS_{F0}, Portugese Subjects, generation F0; PS_{F1}, Portugese subjects, generation F1; TS_{F0}, Toronto subjects, generation F0; TS_{F1}, Toronto subjects, generation F1; UTR, untranslated region.

was 21.1 years. In the Toronto sample, the male:female ratio was 2.3:1, and in the Portuguese sample this ratio was 1.8:1. In the combined sample, the male:female ratio was 1.9:1. None of these differences were statistically significant. Allele and genotype frequencies are reported in Table 1. In the F0 generation, allele frequencies were not significantly different between the Toronto and Portuguese samples at any polymorphism (Table 1). In the F1 generation, only the intron 3 polymorphism showed a significant difference (intron 3: $\chi^2 = 5.095$, $df = 1$, $p = .024$; Table 1). Results from tests of genetic structure are reported in Table 2. The exact tests of population differentiation performed in Arlequin are analogous to a Fisher's Exact Test based on haplotype frequencies (Raymond and Rousset 1995). No significant differentiation between populations was indicated. Gene diversity and genetic structure of the four populations defined above were examined through the 16 possible haplotypes generated from the four polymorphisms. Estimated haplotype frequencies and genetic diversity are presented in Table 3. Genetic structure was examined by calculating molecular variances from pairwise differences between haplotypes, within and among populations. A fixation index (F_{ST}) was then calculated as a ratio of variance components and describes whether the majority of genetic variance is within populations or between them (see Excoffier et al [1992] or Excoffier [2001] for details of the procedure). Pairwise F_{ST} values for the four populations and global F_{ST} values for individual polymorphisms are shown in Table 2. None of the tests indicated a significant difference in genetic structure between populations. Based on these population level comparisons, the samples were combined in the overall analysis.

Genetic Association Analysis

We found a statistically significant genetic association between the STX1A intron 7 polymorphism and schizophrenia with the TDT in the nuclear families ($\chi^2 = 5.898$; $df = 1$; $p = .015$; Table 4), indicating that the C allele was transmitted more frequently to schizophrenia probands than was the T allele. FBAT analysis confirmed this association under an additive, biallelic model ($z = 2.280$; $p = .023$; Table 4). There was no significant genetic association with schizophrenia for any of the other markers under either a TDT analysis or an FBAT analysis (Table 4). In the case-control sample, the intron 7 polymorphism did not show a significant association with schizophrenia ($\chi^2 = 1.82$, $df = 1$, $p = .177$); however, when combined with the TDT score, the results are stronger than the TDT alone ($z_c = 2.859$, $p = .004$; Table 4). This result would still be significant after Bonferroni correction over the four markers studied ($p = .016$). Haplotype analysis of all four STX1A polymorphisms in the family-based sample generated a significant result for the haplotype 1-1-1-1 ($\chi^2 = 3.893$, $df = 1$, $p = .048$); however, the global chi-square for the analysis was not significant (global $\chi^2 = 8.128$, $df = 8$, $p = .421$; Table 5). Several haplotype combinations consisting of two or three of the markers also revealed significant associations, though global chi-squares never achieved significance in any of the analyses (Table 5).

Table 2. Summary of Population Genetic Structure Measures

	Population Pairwise F_{ST} (*p* value)			Global F_{ST} for Individual Polymorphisms		Exact Test of Population Differentiation (*p* value)			
	TS_{F0}	PS_{F0}	TS_{F1}		F_{ST} (*p* value)		TS_{F0}	PS_{F0}	TS_{F1}
PS_{F0}	.035 (.07868)			Exon 3	−.0029 (.32551)	PS_{F0}	$p = .465$		
TS_{F1}	−.003 (.72628)	.0486 (.05719)		Intron 3	−.0071 (.95894)	TS_{F1}	$p = .916$	$p = .394$	
PS_{F1}	−.0091 (.72132)	−.0033 (.31273)	−.0037 (.44793)	Intron 7	.0037 (.15347)	PS_{F1}	$p = .346$	$p = .995$	$p = .098$
				3′ UTR	−.0004 (.40567)				

F0, parental generation (includes aunts and uncles); F1, proband (includes all siblings); F_{ST}, fixation index; PS_{FO}, Portugese subjects, generation F0; PS_{F1}, Portugese subjects, generation F1; TS_{FO}, Toronto subjects, generation F0; TS_{F1}, Toronto subjects, generation F1; UTR, untranslated region.

Table 3. Haplotype Frequencies and Genetic Diversity

	TS_{F0}	TS_{F1}	PS_{F0}	PS_{F1}	All Subjects
Haplotype[a]					
1.1.1.1	.06031	.03872	<1.000e-05	.02477	.04127
1.1.1.2	.01858	.01722	<1.000e-05	<1.000e-05	.01613
1.1.2.1	.05520	.05697	.05556	.09026	.06280
1.1.2.2	.36162	.35801	.22222	.31818	.34809
1.2.1.1	<1.000e-05	<1.000e-05	<1.000e-05	<1.000e-05	<1.000e-05
1.2.1.2	<1.000e-05	.00876	<1.000e-05	<1.000e-05	.00390
1.2.2.1	.00788	<1.000e-05	<1.000e-05	<1.000e-05	.00403
1.2.2.2	.02045	.03396	.05556	.04406	.03195
2.1.1.1	.30561	.27867	.61111	.42978	.31952
2.1.1.2	.00493	.01572	<1.000e-05	<1.000e-05	.00899
2.1.2.1	.08972	.10926	.05556	.04610	.09065
2.1.2.2	.01750	.02998	<1.000e-05	<1.000e-05	.02277
2.2.1.1	<1.000e-05	<1.000e-05	<1.000e-05	<1.000e-05	<1.000e-05
2.2.1.2	<1.000e-05	<1.000e-05	<1.000e-05	<1.000e-05	<1.000e-05
2.2.2.1	.05820	.05274	<1.000e-05	.02273	.04909
2.2.2.2	<1.000e-05	<1.000e-05	<1.000e-05	.02412	.00083
Genetic Diversity[b]	.8450 (+/− .0107)	.8646 (+/− .0074)	.8039 (+/− .0691)	.8457 (+/− .0256)	.8529 (+/− .0056)

F0, parental generation (includes aunts and uncles); F1, proband (includes all siblings); PS_{F0}, Portugese subjects, generation F0; PS_{F1}, Portugese subjects, generation F1; TS_{F0}, Toronto subjects, generation F0; TS_{F1}, Toronto subjects, generation F1.

[a]Haplotype frequencies are estimated using the expectation-maximization algorithm with maximum 5000 iterations and a threshold (epsilon value) of 1.0e7.

[b]Genetic diversity is defined as the probability that two haplotypes chosen at random from the population are different.

Discussion

We initially genotyped two STX1A polymorphisms in a group of nuclear families based on evidence of abnormal synaptic protein and mRNA levels in schizophrenia. After finding a significant association with the intron 7 marker, we subsequently genotyped 2 additional, flanking markers in the nuclear families. We also genotyped the original two markers in an independent case-control sample. In both the family-based and the case-control sample, the C allele (allele 2′) of the intron 7 polymorphism is transmitted significantly more to schizophrenia probands, and the combined results ($p = .004$) are stronger than for the nuclear families alone. Haplotype analyses were performed first using the two original polymorphisms, and subsequently with all four. With the first two polymorphisms, there was a nonsignificant overtransmission of allele 2′ to schizophrenia probands. When haplotype analyses were extended to include all combinations of 2, 3, or 4 of the polymorphisms, a pattern emerged in which significant haplotypes all contained the 1′ allele of each polymorphism. Specific to the intron 7 polymorphism, each significant haplotype showed a decreased transmission of allele 1′ compared to what was expected. Furthermore, most haplotypes in which the intron 7 C allele (allele 2′) was present with allele 1′ of the other polymorphisms, showed an increased transmission over what was expected, although these measures did not achieve significance (data not shown). To interpret the pattern of these findings, it would be useful to know the age and origin of each polymorphism; however, we were limited by the available data to measures of LD.

Tests of concordance with Hardy-Weinberg Equilibrium (HWE) consistently showed a significant departure from HWE for the exon 3 polymorphism. This was found for the F1 (proband) generation in tests using the Toronto and Portuguese samples by themselves, and in the combined sample set, ($p = .036$, $p = .043$, and $p = .002$ respectively). The TS_{F0} population also showed a significant departure from HWE for the exon 3 polymorphism (p

Table 4. Uncorrected Chi-Squared Values for Association Tests

	Case-Control		TDT			FBAT		
	χ^2 1 *df* (*p* value)	Combined[a] z_c (*p* value)	Transmissions (no.) (b/c)[b]	χ^2 (1*df*)	*p*	Informative Families (no.)	*z*-Score	*p*
Exon 3	1.553 (.213)	.920 (.358)	10/18	2.286	.131	36	.636	.525
Intron 3	–	–	10/13	.391	.532	23	.090	.929
Intron 7	1.82 (.177)	2.859 (.004)	16/33	5.898	.015	60	2.280	.023
3′ UTR	–	–	32/29	.148	.700	68	.387	.699

FBAT, family-based association test; TDT, transmission disequilibrium test; UTR, untranslated region.

[a]Chi-square values for case-control and TDT analyses were converted to *z* scores, and combined using the inverse normal method.

[b]Indicates the number of times allele one is transmitted (b) and not transmitted (c) to affected offspring in informative families.

Table 5. Significant Associations Revealed from Haplotype Analyses[a]

Markers[b]	Haplotype	Observed/Expected Values	χ^2 (1 *df*)	*p*	Global Chi-Square[c] (*df*)	*p*
I; II	1.1	23.141 / 29.149	5.9834	.014	7.5725,(3)	.056
II; III	1.1	215.56 / 226.01	3.1909	.074	4.0719,(3)	.254
I; II; III	1.1.1	27.691 / 34.563	8.0068	.005	10.42,(6)	.108
I; II; IV	1.1.1	21.497 / 25.998	4.3335	.037	6.9615,(6)	.324
I; II; III; IV	1.1.1.1	21.722 / 25.997	3.8932	.048	8.1288,(8)	.421

[a]Marker order: exon 3. intron 3. intron 7. 3′ untranslated region (UTR).
[b]Polymorphisms: I. exon 3; II. intron 3; III. intron 7; IV. 3′ UTR.
[c]Global chi-square is calculated from all haplotypes with frequency >3% in the analysis from which the reported association is taken.

= .005), although this was not the case for the PS_{F0} nor for the combined F0 populations. Tests for concordance with HWE for the other polymorphisms were consistently nonsignificant in any population studied. The shift from concordance to nonconcordance with HWE in the Portuguese F1 generation is an indicator that this subpopulation does not represent a population that has resulted from random mating in the previous generation. Whether such a development is an indicator of selection among subjects that are susceptible to mental illness, a consequence of subsampling families from a larger population based on a specific criteria (a child who has developed schizophrenia), or a consequence of overlapping generations or reflects some other factor in the population's history is not yet clear. In any case, the finding is not supported by association tests, and for now is best treated as a condition on LD measures.

The exon 3 polymorphism is in LD with the intron 7 and 3′UTR polymorphisms, but not with the intron 3 polymorphism (Table 6). These results are not consistent with the HWE tests, and so the previous finding will be considered an artifact of the sampling process and not an indicator of a relationship with the schizophrenia phenotype. Likewise, the intron 3 and 3′UTR polymorphisms are not in LD with each other (Table 6). The intron 7 polymorphism is in LD with all other polymorphisms in the Toronto and combined populations; however, in the Portuguese population, in both the F0 and F1 generations, intron 7 is not in LD with intron 3 (Table 6). With reference to the haplotype analysis, the strong LD between intron 7 and the other polymorphisms supports the idea that the associations are being driven by the intron 7 polymorphism. Indeed, the haplotype generated from intron 7 and intron 3 (which are not in LD with each other) shows a trend, but is not significant at the 95% level.

The syntaxin 1A gene has been mapped to chromosome region 7q11.2 (Nakayama et al 1997), which has not been previously linked to schizophrenia in whole genome scans (Riley and McGuffin 2000). The chromosome 7q21.1-q21.3 (Blouin et al 1998) and 7q21.3-q22 (Faraone et al 1998) regions have been linked to schizophrenia but are quite far from the STX1A gene; however, there is evidence that STX1A may affect behavior in disorders such as Williams syndrome (WS). WS is a neurodevelopmental, chromosomal-deletion disorder that may involve the STX1A gene region (Botta et al 1999b; Nakayama et al 1998), although recent evidence suggests that the STX1A gene is not always deleted (Botta et al 1999a; Tassabehji et al 1999; Wu et al 2002). Clinical features of the syndrome include: dysmorphic facies, mental retardation or learning difficulties, elastin arteriopathy, a unique cognitive profile of relative strength in auditory rote memory and language and extreme weakness in visuospatial constructive cognition, and a typical personality that includes overfriendliness, anxiety, and attention problems (Morris and Mervis 2000). Considering the phenotype of WS, it is possible that mutation or altered expression of the STX1A gene, rather than deletion, may contribute to the cognitive or psychological impairments seen in schizophrenia or that abnormal synaptic function may contribute to altered dopamine release and thus result in psychotic symptoms.

We have presented data that suggest an association between an intron 7 polymorphism and schizophrenia in probands collected from the Toronto area and from Portugal in two independent samples: case-control and nuclear families; however, replication of these findings and further studies into the functional impact of this STX1A polymorphism are required. The most compelling evidence of STX1A involvement would be the demonstration of a pathophysiologic mechanism in addition to an association between the gene and the illness.

Table 6. Linkage Disequilibruim Values for Pairs of Polymorphisms

Pair	F0	F1	TS_{F0}	PS_{F0}	TS_{F1}	PS_{F1}
Exon 3-Intron 3	$p = .591$	$p = .544$	$p = .463$	$p = .549$	$p = .353$	$p = .455$
Exon 3-Intron 7	$p < .001$	$p < .001$	$p < .001$	$p = .004$	$p < .001$	$p < .001$
Exon 3-3′ UTR	$p < .001$	$p < .001$	$p < .001$	$p = .004$	$p < .001$	$p < .001$
Intron 3-Intron 7	$p < .001$	$p = .008$	$p < .001$	$p = .677$	$p = .013$	$p = .654$
Intron 3-3′ UTR	$p = .762$	$p = .167$	$p = .637$	$p = .161$	$p = .231$	$p = .633$
Intron 7-3′ UTR	$p < .001$	$p < .001$	$p < .001$	$p = .016$	$p < .001$	$p < .001$

F0, parental generation (includes aunts and uncles); F1, proband (includes all siblings); PS_{F0}, Portugese subjects, generation F0; PS_{F1}, Portugese subjects, generation F1; TS_{F0}, Toronto subjects, generation F0; TS_{F1}, Toronto subjects, generation F1; UTR, untranslated region.

We acknowledge the support of the Canadian Institutes for Health Research (CIHR), the National Institutes of Mental Health, and the Ontario Mental Health Foundation. AHCW holds a CIHR Clinician-Scientist Fellowship. HHMVT holds a Canada Research Chair in Neurobiology. WGH was supported by a CIHR Scientist Award and CIHR NET 54013. This research would not have been possible without the participation of patients with schizophrenia, their families, and control subjects, and we are grateful for their generosity.

Both senior authors HHMVT and JLK contributed equally to this project.

American Psychiatric Association (1994): *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. Washington, DC: American Psychiatric Association Press.

Barr AM, Song C, Sawada K, Young CE, Honer WG, Phillips AG (2003): Tolerance to the anhedonic effects of lipopolysaccharide is associated with changes in syntaxin immunoreactivity in the nucleus accumbens. *Int J Neuropsychopharmacol* 6:23–34.

Bennett MK, Calakos N, Scheller RH (1992): Syntaxin: A synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. *Science* 257:255–259.

Blouin JL, Dombroski BA, Nath SK, Lasseter VK, Wolyniec PS, Nestadt G, et al (1998): Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. *Nat Genet* 20:70–73.

Botta A, Novelli G, Mari A, Novelli A, Sabani M, Korenberg J, et al (1999a): Detection of an atypical 7q11.23 deletion in Williams syndrome patients which does not include the STX1A and FZD3 genes. *J Med Genet* 36:478–480.

Botta A, Sangiuolo F, Calza L, Giardino L, Potenza S, Novelli G, et al (1999b): Expression analysis and protein localization of the human HPC-1/syntaxin 1A, a gene deleted in Williams syndrome. *Genomics* 62:525–528.

Clayton D, Jones H (1999): Transmission/disequilibrium tests for extended marker haplotypes. *Am J Hum Genet* 65:1161–1169.

Excoffier L (2001): Analysis of population subdivision. In Balding D, Bishop M, Cannings C, editors. *Handbook of Statistical Genetics.* New York: Wiley and Sons Ltd, 271–308.

Excoffier L, Smouse PE, Quattro JM (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131:479–491.

Faraone SV, Matise T, Svrakic D, Pepple J, Malaspina D, Suarez B, et al (1998): Genome scan of European-American schizophrenia pedigrees: Results of the NIMH Genetics Initiative and Millennium Consortium. *Am J Med Genet* 81:290–295.

Gabriel SM, Haroutunian V, Powchik P, Honer WG, Davidson M, Davies P, et al (1997): Increased concentrations of presynaptic proteins in the cingulate cortex of subjects with schizophrenia. *Arch Gen Psychiatry* 54:559–566.

Hedges LV, Olkin I, Ingram O (1985): Statistical methods for meta-analysis. London: Academic Press, 39.

Honer WG, Falkai P, Young C, Wang T, Xie J, Bonner J, et al (1997): Cingulate cortex synaptic terminal proteins and neural cell adhesion molecule in schizophrenia. *Neuroscience* 78:99–110.

Lahiri DK, Nurnberger JI Jr (1991): A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 19:5444.

Laird NM, Horvath S, Xu X (2000): Implementing a unified approach to family-based tests of association. *Genet Epidemiol* 19:S36–42.

Maxwell ME (1992): *Family Interview for Genetic Studies (FIGS): Manual for FIGS*. Bethesda, MD: Clinical Neurogenetics Branch, Intramural Research Program. National Institute of Mental Health.

Morris CA, Mervis CB (2000): Williams syndrome and related disorders. *Annu Rev Genomics Hum Genet* 1:461–484.

Mukaetova-Ladinska EB, Hurt J, Honer WG, Harrington CR, Wischik CM (2002): Loss of synaptic but not cytoskeletal proteins in the cerebellum of chronic schizophrenics. *Neurosci Lett* 317:161–165.

Nakayama T, Fujiwara T, Miyazawa A, Asakawa S, Shimizu N, Shimizu Y, et al (1997): Mapping of the human HPC-1/syntaxin 1A gene (STX1A) to chromosome 7 band q11.2. *Genomics* 42:173–176.

Nakayama T, Matsuoka R, Kimura M, Hirota H, Mikoshiba K, Shimizu Y, et al (1998): Hemizygous deletion of the HPC-1/syntaxin 1A gene (STX1A) in patients with Williams syndrome. *Cytogenet Cell Genet* 82:49–51.

Nurnberger JI Jr, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J, et al (1994): Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative. *Arch Gen Psychiatry* 51:849–59 ; discussion:863–864.

Quick MW (2002): Role of syntaxin 1A on serotonin transporter expression in developing thalamocortical neurons. *Int J Dev Neurosci* 20:219–224.

Raymond M, Rousset F (1995): An exact test for population differentiation. *Evolution* 49:1280–1283.

Riley BP, McGuffin P (2000): Linkage and associated studies of schizophrenia. *Am J Med Genet* 97:23–44.

Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, Zhivotovsky LA, et al (2002): Genetic structure of human populations. *Science* 298:2381–5.

Schneider S, Roessli D, Excoffier L (2000): *Arlequin version 2.0: A software for population genetics data analysis, 2.0 ed*. Geneva: Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Slatkin M, Excoffier L (1996): Testing for linkage disequilibrium in genotypic data using the Expectation-Maximization algorithm. *Heredity* 76:377–383.

Sokolov BP, Tcherepanov AA, Haroutunian V, Davis KL (2000): Levels of mRNAs encoding synaptic vesicle and synaptic plasma membrane proteins in the temporal cortex of elderly schizophrenic patients. *Biol Psychiatry* 48:184–196.

Sollner T, Bennett MK, Whiteheart SW, Scheller RH, Rothman JE (1993): A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. *Cell* 75:409–418.

Spielman RS, Ewens WJ (1998): A sibship test for linkage in the presence of association: The sib transmission/disequilibrium test. *Am J Hum Genet* 62:450–458.

Spielman RS, McGinnis RE, Ewens WJ (1993): Transmission test for linkage disequilibrium: The insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506–516.

Sung U, Apparsundaram S, Galli A, Kahlig KM, Savchenko V, Schroeter S, et al (2003): A regulated interaction of syntaxin 1A with the antidepressant-sensitive norepinephrine transporter establishes catecholamine clearance capacity. *J Neurosci* 23:1697–1709.

Tassabehji M, Metcalfe K, Karmiloff-Smith A, Carette MJ, Grant J, Dennis N, et al (1999): Williams syndrome: Use of chromosomal microdeletions as a tool to dissect cognitive and physical phenotypes. *Am J Hum Genet* 64:118–1125.

Tsunoda K, Sanke T, Nakagawa T, Furuta H, Nanjo K (2001): Single nucleotide polymorphism (D68D, T to C) in the syntaxin 1A gene correlates to age at onset and insulin requirement in Type II diabetic patients. *Diabetologia* 44:2092–2097.

Wu YQ, Bejjani BA, Tsui LC, Mandel A, Osborne LR, Shaffer LG (2002): Refinement of the genomic structure of STX1A and mutation analysis in non-deletion Williams syndrome patients. *Am J Med Genet* 109:121–124.