The Type 2 Diabetes Mellitus Susceptibility Gene *IGF2BP2* Is Associated With Schizophrenia in a Han Chinese Population

Xuan Zhang, PhD; Li Hui, PhD; Yang Liu, PhD; Zhen-Qi Wang, PhD; Yang You, MD; Li-ning Miao, MD, PhD; Shi-Long Sun, PhD; Song-Lei Guan, PhD; Yutao Xiang, MD, PhD; Thomas R. Kosten, MD; and Xiang Yang Zhang, MD, PhD

ABSTRACT

Background: Patients with schizophrenia have an increased prevalence of type 2 diabetes mellitus, and type 2 diabetes mellitus has shown an association with the rs4402960 gene polymorphism in the insulin-like growth factor II messenger RNA (mRNA)–binding protein 2 gene (*IGF2BP2*). We tested this polymorphism and mRNA expression levels of *IGF2BP2* for an association in Han Chinese patients with schizophrenia compared to healthy controls.

Method: The rs4402960 polymorphism was genotyped in 790 chronic schizophrenic patients (diagnosed according to *DSM-IV*) and 1,083 unrelated healthy controls in a case-control design. The *IGF2BP2* gene expression levels were assayed in 34 patients with chronic schizophrenia and 30 healthy controls by using real-time polymerase chain reaction (PCR). The study was conducted between 2005 and 2007.

Results: We found significant differences in the rs4402960 genotype ($\chi^2_2 = 7.316$, $P = .026$) and allele ($\chi^2_1 = 7.056$, $P = .008$) distributions between the patient and control groups. The rs4402960 T allelic frequency was significantly higher in male schizophrenic patients than male controls (28.9% vs 23.5%; $P = .004$) but not in female patients compared to female controls (27.1% vs 25.5%; $P = .498$). When real-time PCR was used, the *IGF2BP2* gene's isoform B expression levels were significantly greater in schizophrenia than controls ($P = .0008$).

Conclusions: These results suggest that the *IGF2BP2* gene may play a role in susceptibility to schizophrenia, supporting the hypothesis that the co-occurrence of type 2 diabetes mellitus and schizophrenia may be explained by shared genetic risk variants. However, this finding remains preliminary since this association has yet to be replicated.

J Clin Psychiatry 2013;74(4):e287–e292

Submitted: *April 11, 2012; accepted October 25, 2012 (doi:10.4088/JCP.12m07846).*

Corresponding author: *Xiang Yang Zhang, MD, PhD, Jilin University, 18 Ziqiang St, Changchun 130041, China (xyzhang@bcm.edu).*

The prevalence of type 2 diabetes mellitus has been reported as 2 to 4 times higher in people with schizophrenia, including people with schizophrenia in Taiwan, than in the general population across different ethnic groups.[2–4] A recent study suggested that type 2 diabetes mellitus is more common among male patients with schizophrenia,[5] but the reasons for any association are most likely to be multifactorial, including induction by second-generation antipsychotics.[2,6–9] However, Lin and Shuldiner[3] proposed shared genetic risk variants that exert pleiotropic effects (ie, the same DNA sequence causing both phenotypes of schizophrenia and abnormal glucose metabolism).[3,6,10] Additional support for pleiotropism includes an elevated risk of type 2 diabetes mellitus among drug-naive or first-episode patients with schizophrenia and among their relatives.[11–15] Moreover, studies from the pre-neuroleptic era suggest that the comorbidity exists independent of antipsychotic treatment and supports shared risk factors between schizophrenia and diabetes.[16–18]

Insulin-like growth factor II messenger RNA (mRNA)–binding proteins 1, 2, and 3 (IMP1, IMP2, and IMP3) belong to a family of ribonucleic acid (RNA)–binding proteins implicated in mRNA localization, turnover, and translational control.[19] Recent studies suggested a specific role for insulin-like growth factor II mRNA–binding protein 2 gene (*IGF2BP2*) or *IMP2*, but not for the other members of IMP family, in the development of type 2 diabetes mellitus.[20] Polymorphisms of *IGF2BP2* are associated with impaired pancreatic β-cell function, including a lower fasting insulin concentration and reduced glucose-stimulated insulin secretion.[21] Several important single nucleotide polymorphisms (SNPs) have been identified in the *IGF2BP2* gene. Among several variants identified in *IGF2BP2,* the rs4402960 polymorphism with a G→T substitution in intron 2 has attracted the most attention for its predisposition to type 2 diabetes mellitus. Subsequent studies have independently replicated this association, and the most recent meta-analysis demonstrated that this common polymorphism has an overall random-effects per allele odds ratio (OR) of 1.13 (95% CI, 1.12–1.15; $P < 10^{-5}$) for type 2 diabetes mellitus.[22,23]

Thus, it is reasonable to hypothesize that the *IGF2BP2* gene is a potential candidate gene contributing to the comorbidity between schizophrenia and type 2 diabetes mellitus. To our knowledge, however, the association between the *IGF2BP2* gene and schizophrenia has not been investigated. Therefore, in the present study, we compared (1) the rates of this SNP (rs4402960) in the *IGF2BP2* gene between schizophrenia and controls in a Chinese population and (2) the expression of the 2 *IGF2BP2* mRNA isoforms in the peripheral blood lymphocytes of schizophrenia to controls, since the human *IGF2BP2* gene, which is localized on chromosome 3q27.2, can be expressed in lymphocytes, although it is more highly expressed in other tissues (http://biogps.org/). The human *IGF2BP2* gene has 2 mRNA isoforms: isoform A and isoform B. Isoform A (NM_006548.4; spanning 3.676 kb) has 1 more exon than isoform B (NM_001007225.1; spanning 3.547 kb) (http://www.ncbi.nlm.nih.gov/).

Clinical Points

- Patients with schizophrenia have an increased prevalence of type 2 diabetes mellitus, and type 2 diabetes mellitus has shown an association with the rs4402960 polymorphism of the insulin-like growth factor II mRNA–binding protein 2 gene (*IGF2BP2*).
- The rs4402960 polymorphism was found to be associated with vulnerability to schizophrenia, and the *IGF2BP2* gene's expression levels were significantly greater in schizophrenia.
- The co-occurrence of type 2 diabetes mellitus and schizophrenia may be caused by shared genetic risk variants.

METHOD

Subjects

We approached all inpatients in the Changchun Kaixuan Hospital, a psychiatric hospital in Jilin Province, China, in the period between 2005 and 2007, using a cross-sectional naturalistic design. The recruitment criteria included Han Chinese inpatients who (1) were aged 25–75 years, (2) were confirmed to have a diagnosis of schizophrenia according to *DSM-IV* criteria, (3) had at least 2 years of illness, (4) were receiving stable doses of oral antipsychotic drugs for at least 6 months before entry into the study, and (5) provided written informed consent and were able to take part in their psychopathological assessment. We enrolled 790 chronic medicated schizophrenia inpatients (men, n = 417; women, n = 373). Two independent, experienced psychiatrists diagnosed and clinically assessed these patients following a clinical interview. The mean ± SD age of the patients was 36.7 ± 12.5 years. All patients were of the chronic type and had been ill for a mean ± SD of 11.7 ± 6.6 years.

We recruited 1,083 age- and gender-matched controls (men, n = 709; women, n = 374) from the local community through advertisement. The mean ± SD age of the controls was 36.9 ± 11.1 years. A clinical psychiatrist assessed current mental status and personal or family history of any mental disorder and found no psychiatric disorders among the healthy control subjects.

For the real-time quantitative polymerase chain reaction (PCR) analysis, we collected peripheral leukocyte samples from 34 Han Chinese subjects with chronic schizophrenia with a mean ± SD age of 30.3 ± 5.9 years (18 men and 16 women) and 30 healthy controls with a mean ± SD age of 31.9 ± 5.4 years (16 men and 14 women). All the subjects with chronic schizophrenia had 2 or more episodes of psychosis and were treated with antipsychotic medication when the blood samples were collected.

A complete medical history and physical examination were obtained from patients and control subjects. Subjects with severe physical diseases were excluded. Participants were not dependent on alcohol or other substances other than tobacco, based on their self-report.

All the subjects were Chinese of Han descent and originally came from the Northeast area of China. They gave informed consent to participate in the genetic research, which was approved by the ethics committee of Jilin University, Changchun, China.

Clinical Assessment

The psychotic symptoms under study included the following 14 items scored as present or absent: auditory hallucinations, incoherent thinking, illogical thought, bizarre behavior, aggressive behavior, and delusions of observation, influence, sin, negation, being revealed, persecution, jealousy, grandeur, and love. An overall hallucination-delusion syndrome was also scored as present or absent. Three negative symptoms (alogia, affective flattening, and avolition) were assessed as quantitative traits by using the Scale for the Assessment of Negative Symptoms (SANS).[24] These 3 negative symptoms were scored as 4 grades: 0 for absent, 1 for questionable, 2 for mild or moderate, and 3 for marked or severe.

Genotyping

Genomic DNA used for PCR amplification was extracted from whole blood samples by using a DNA extraction kit (Promega, Beijing, China). The rs4402960 polymorphism was amplified by a PCR based on the SNP map of the *IGF2BP2* gene (http://www.ncbi.nlm.nih.gov/SNP). Using the Primer 3 software,[25] we designed the following primers: the upstream primer, 5′-TGGAACCCTGGTATTCCAAGATT-3′ and the downstream primer, 5′-TATCTGTGGCATGTTTG CATACACAATCAGTATTT-3′. The conditions used for PCR amplification included an initial denaturation at 95°C for 5 minutes, followed by 35–40 cycles of 94°C for 45 seconds, 55–60°C for 45 seconds, and 72°C for 45 seconds, and a final elongation at 72°C for 10 minutes. The product of 137 base pairs (bp) was digested with Dra I (Promega, Madison, Wisconsin) at 37°C overnight, yielding fragments of 101 and 36 bp in the presence of the –T allele or the single uncut fragment in the presence of the –G allele.

A research assistant who was blinded to the clinical status genotyped every subject twice for accuracy of genotyping. In each experiment, an individual known to be heterozygous for rs4402960 polymorphism was included as a positive control to ensure amplification of both alleles. A reagent control (BanDing Biomedical Inc, Beijing, China) without DNA served as a negative control. Negative and positive controls were included in each run. Also, a subsample (n = 50) of the genotyped samples was randomly selected for duplication accuracy with a direct sequencing protocol (SinoGenoMax, Beijing, China), and reproducibility showed perfect concordance that was routinely > 0.99. In addition, the DNA samples showed genotype call rates of more than 0.99, which passed the quality criterion for genotyping (greater than 0.95).

RNA Preparation and Real-Time Quantitative PCR Analysis

Primer and probe design. Primers and probes were designed by using the Primer 3 on-line tool.[25] Since humans have 2 isoforms of mRNA, isoform A (NM_006548.4) and

Table 1. Primer Sequence for Real-Time Quantitative Polymerase Chain Reaction

Primer Name	Sequence (5′ to 3′)	Product Length (base pairs)
IGF2BP2-F1	gtcagcgaaaggatggtca	138
IGF2BP2-R1	cactctgatatgcgcttcca	
IGF2BP2-F2	ccggaaagaaccatcactgt	133
IGF2BP2-R2	ctgggatcagattggcttgt	
IGF2BP2-F2	ccggaaagaaccatcactgt	129
IGF2BP2-R3	gaagtatccggagtgggtgt	
GAPDH-F	gcaccgtcaaggctgagaac	138
GAPDH-R	tggtgaagacgccagtgga	

isoform B (NM_001007225.1), we designed 3 specific primers for PCR amplification. The amplicon 1 primer corresponded to the target sequence of both isoform A and isoform B. The amplicon 2 primer corresponded to the sequence of isoform A, and the amplicon 3 primer corresponded to the sequence of isoform B. We used human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA expression to normalize the *IGF2BP2* mRNA expression levels as an RNA internal control. Primers and probes are shown in Table 1 as synthesized by Sangon Biotech Co Ltd (Shanghai, China).

Real-time quantitative PCR analysis. A 5-mL peripheral blood sample was collected between 6 AM and 8 AM following an overnight fast. Total RNA was extracted with TRIZOL Reagent (Invitrogen, Carlsbad, California) from leukocytes isolated from the fresh blood samples and treated with DNase (NEB, Ipswich, Massachusetts) according to the manufacturer's instructions. Complementary DNA was synthesized by using TaKaRa RNA PCR Kit (AMV) Ver 3.0, DRR019A (Takara Bio Inc, Dalian, China) with a random primer. We assayed RNA concentration and purity using a SmartSpec Plus spectrophotometer (Bio-Rad Laboratories, Hercules, CA). We measured *IGF2BP2* mRNA expression levels using Mx3000P real-time PCR (Stratagene, Santa Clara, California) with SYBR green I fluorescence (Takara Bio Inc, Dalian, China).

Following synthesis, complementary DNA reaction mixtures were diluted up to 50 μL with nuclease-free water and stored at −20°C until analysis. Quantitative PCR reactions consisted of SYBR Premix Ex Tag (Takara Bio Inc) (2×) 12.5 μL, ROX reference Dye II (Takara Bio Inc) (50×) 0.5 μL, 1.0 μL of each primer and probe (10 μM), and 2 μL complementary DNA reaction mixture (0.1 μg of RNA equivalent) in a total 25 μL reaction volume. All samples were assayed on the Mx3000P real-time PCR (Stratagene) by using the following cycling conditions: 95°C 30 seconds→(95°C 20 seconds→60°C 35 seconds) × 45 cycles→(95°C 1 minute→55°C 30 seconds→95°C 30 seconds) × 1 cycle, monitoring fluorescence at the green and yellow channels.

Experiments were performed with duplicates for each sample. Results of the real-time PCR data were represented as comparative threshold cycle (C_t) values, where C_t was defined as the threshold cycle of PCR at which the amplified product was first detected. We used a comparative C_t method processed by SDS software version 2.2 (Applied Biosystems, Carlsbad, California). The C_t value of each sample was obtained by subtracting the mean *GAPDH* C_t value of each sample from the average *IGF2BP2* C_t value of each sample. The equation $2^{-\Delta\Delta C_t}$ was used to represent the expression level of each sample (Applied Biosystems).

Statistical Analysis

Hardy-Weinberg equilibrium analysis was performed with the program Haploview 4.1.[26] The UNPHASED program (version 3.0.13)[27] (http://unphased.sourceforge.net) was applied to analyze genotyping data in a phased unknown model. An OR value and 95% confidence interval (CI) were automatically calculated by the UNPHASED program. Group differences were compared by using the independent samples *t* test or 1-way analysis of variance for continuous variables and χ^2 for categorical variables by using SPSS 14.0 for Windows (SPSS Inc, Chicago, Illinois).

The power of the sample was calculated with Quanto Software,[28] with known risk allele frequencies, a schizophrenia population prevalence of 0.01, and examination of log additive, recessive, and dominant models. All the tests were 2-tailed, and the significance level was set at a *P* value of .05.

RESULTS

Association Analyses for *IGF2BP2*

The χ^2 goodness-of-fit test showed that the genotypic distributions of the *IGF2BP2* rs4402960 polymorphism in both case and control groups were consistent with Hardy-Weinberg equilibrium (both *P* values > .05).

Table 2 shows significant differences in the rs4402960 genotype ($\chi^2_2 = 7.316$, $P = .026$) and allele ($\chi^2_1 = 7.056$, $P = .008$; OR = 1.221; 95% CI, 1.054–1.415) distributions between the patient and control groups. In men, we found a significant association between the rs4402960 polymorphism and schizophrenia, both at the genotypic ($\chi^2_2 = 8.39$, $P = .015$) and at the allelic level ($\chi^2_1 = 8.08$, $P = .004$; OR = 1.324, 95% CI, 1.091–1.607). In women, we found no significant association between the rs4402960 polymorphism and schizophrenia at the genotypic ($\chi^2_2 = 4.35$, $P = .11$) and allelic level ($\chi^2_1 = 0.46$, $P = .50$). In addition, there were no genotype differences in terms of number of years of illness ($P > .05$). Also, Table 3 shows no significant associations between the rs4402960 genotype and any clinical symptoms (all *P* values > .05).

This total sample had 0.99 statistical power to detect a polymorphism associated with schizophrenia through dominant, recessive, or log-additive inheritance, with an OR of 2 ($\alpha = .05$, 2-tailed test). However, for those association analyses, only the dominant model was used.

Real-Time Quantitative PCR

The equations for the standard curve, coefficient of regression, and amplification efficiency are shown in Table 4. With *GAPDH* mRNA expression as internal healthy control, the *IGF2BP2* mRNA expression levels are shown in Table 5. The real-time PCR showed no difference in total expression (isoform A + isoform B) or isoform A expression levels between the schizophrenia and healthy control

Table 2. Comparison of the *IGF2BP2* rs4402960 Genotype and Allele Frequencies Between Schizophrenia (n = 790) and Healthy Controls (n = 1,083)

	Allelic Frequency				Genotypic Frequency				
Group	T (%)	G (%)	χ^2	*P*	T/T (%)	T/G (%)	G/G (%)	χ^2	*P*
Total									
Case	443 (28.0)	1,137 (72.0)	7.05	.008	65 (8.2)	313 (39.6)	412 (52.2)	7.32	.026
Control	524 (24.2)	1,642 (75.8)			62 (5.7)	400 (36.9)	621 (57.3)		
Male									
Case	241 (28.9)	593 (71.1)	8.08	.004	35 (8.4)	171 (41.0)	211 (50.6)	8.39	.015
Control	333 (23.5)	1,085 (76.5)			45 (6.3)	243 (34.3)	421 (59.4)		
Female									
Case	202 (27.1)	544 (72.9)	0.46	.498	30 (8.0)	142 (38.1)	201 (53.9)	4.35	.114
Control	191 (25.5)	557 (74.5)			17 (4.5)	157 (42.0)	200 (53.5)		

Table 3. Genotypic Association Between rs4402960 and Psychotic Symptoms in Schizophrenia Patients

Symptom	χ^2	*df*	*P*
Positive			
Genuine auditory hallucination	0.419	2	.811
Delusion of observation	4.108	2	.128
Delusion of influence	1.759	2	.415
Delusion of sin	0.263	2	.877
Delusion of negation	0.565	2	.754
Delusion of being revealed	0.071	2	.965
Delusion of persecution	2.744	2	.254
Delusion of jealousy	1.613	2	.447
Delusion of grandeur	2.052	2	.358
Delusion of being loved	0.448	2	.799
Incoherence of thinking	1.027	2	.598
Illogic thought	3.163	2	.206
Bizarre behavior	0.960	2	.619
Aggressive behavior	0.820	2	.664
Hallucination-delusion syndrome	0.222	2	.895
Negative			
Poverty of thought	5.755	6	.451
Emotional blunting/apathy	4.377	6	.626
Abulia	4.300	6	.636

subjects. However, the isoform B expression (mean ± SD) was significantly higher in schizophrenia than in controls (1.136 ± 0.324 vs 0.885 ± 0.227, $t = 3.54$, $P = .0008$). Still, there was no significant gender difference in isoform B in both patient (1.106 ± 0.355 for men vs 1.174 ± 0.288 for women; $t = -0.61$, $P = .55$) and control groups (0.844 ± 0.259 for men vs 0.926 ± 0.190 for women; $t = -0.66$, $P = .52$). Furthermore, compared to their corresponding control subjects, male and female patients showed higher isoform B expression (both *P* values < .05).

DISCUSSION

This study found that the rs4402960 polymorphism of the *IGF2BP2* gene may be involved in susceptibility to schizophrenia for our Chinese sample, as it is involved in susceptibility to type 2 diabetes mellitus.[23] Our sample size provided high statistical power. Furthermore, we also found increased isoform B expression of mRNA from the *IGF2BP2* gene in peripheral blood samples of schizophrenic patients when we used real-time quantitative PCR analysis. Taken together, our results suggest that *IGF2BP2* may provide some contribution to the comorbidity link between schizophrenia and type 2 diabetes mellitus. However, the association between the rs4402960 polymorphism of the *IGF2BP2* gene and schizophrenia was relatively weak, with ORs of 1.22 for the whole patient group and 1.32 for male patients.

Following the first genome-wide association reports by Saxena et al[29] and Scott et al[30] that the *IGF2BP2* gene variants were associated with type 2 diabetes mellitus, a large number of studies in various populations have replicated that *IGF2BP2* is a type 2 diabetes mellitus susceptibility gene.[21] Several meta-analyses[22,23,31] further supported this robust finding. Our current study showed that a polymorphism within *IGF2BP2* was also associated with increased risk for schizophrenia.

Patients with schizophrenia have shown an increased risk of type 2 diabetes mellitus, and this risk may be greater in men.[1–3,5] This elevated risk of type 2 diabetes mellitus occurs even among drug-naive or first-episode patients with schizophrenia and among their relatives.[11–15,32–34] Interestingly, a recent study showed that 1 type 2 diabetes mellitus at-risk allele located in *TCF7L2*, rs7903146 [T], was associated with schizophrenia in the discovery sample and also in the replication sample from a Danish population, suggesting that the observed comorbidity is partially caused by genetic risk variants.[35] Another recent study[18] also found a significant association of schizophrenia with the *TCF7L2* gene intronic SNP, rs12573128, independently supporting previous findings regarding a possible role of *TCF7L2* in susceptibility to schizophrenia. Taken together, these findings support the proposal that the co-occurrence of type 2 diabetes mellitus and schizophrenia may be caused by shared genetic risk variants. In addition, a shared genetic risk factor for comorbidity of schizophrenia with type 2 diabetes mellitus and other glucose metabolism abnormalities may be enhanced by treatment with antipsychotic medication.[3,9] Hence, it is possible that genetic risk factors for type 2 diabetes mellitus could compound the problem of antipsychotic-induced diabetes through an additive effect of genetic risk factors and treatment risk factors or even through a multiplicative effect in which antipsychotic treatment interacts with genes important in type 2 diabetes mellitus disease pathways.[36] In addition, a higher risk of type 2 diabetes mellitus in men would be consistent with our finding of the genetic association in male but not female schizophrenia.[5] However, our current study cannot distinguish whether *IGF2BP2* may contain independent variants for both disorders or 1 variant that

Table 4. The Equations of Standard Curve, Coefficient of Regression, and Amplification Efficiency

Gene	Equations of Standard Curve	Coefficient of Regression	Amplification Efficiency, %
GAPDH-F/R	y = −3.586 log(x) + 19.24	0.999	90.0
IGF2BP2-F1/R1 (isoform A + isoform B)	y = −3.508 log(x) + 27.79	1.000	92.8
IGF2BP2-F2/R2 (isoform A)	y = −3.640 log(x) + 28.95	0.999	88.2
IGF2BP2-F2/R3 (isoform B)	y = −3.576 log(x) + 29.56	1.000	90.4

Table 5. The Expression of *IGF2BP2* Gene by Real-Time Quantitative Polymerase Chain Reaction[a]

Gene Expression	n	$2^{-\Delta\Delta C_t}$, Mean ± SD	*t*	*df*	*P*
Isoform A + isoform B					
Case	34	1.111 ± 0.479	1.347	62	.183
Control	30	0.980 ± 0.245			
Isoform A					
Case	34	1.130 ± 0.513	1.416	62	.162
Control	30	0.985 ± 0.237			
Isoform B					
Case	34	1.136 ± 0.324	3.540	62	.0008
Control	30	0.885 ± 0.227			

[a]*GAPDH* messenger RNA expression was used as internal healthy control.

exerts a pleiotropic effect causing the 2 different pathological conditions.[18]

In support of the *IGF2BP2* gene's relationship to schizophrenia, we found its increased isoform B expression in peripheral blood samples from our schizophrenic patients. The *IGF2BP2* gene regulates transcription of insulin-like growth factor II, and its overexpression reduces first-phase insulin secretion and disposition index during hyperglycemic clamps.[37–40] A reduced disposition index reflects the failing adaptive capacity and reduced sensitivity of pancreatic β cells to a reduction in insulin levels.[40] Hence, *IGF2BP2* may play an important role in pancreatic β cells' insulin secretion. We hypothesize that increased expression of the *IGF2BP2* gene found in schizophrenic patients from our current study may lead to decreased insulin secretion and pancreatic β cell function in these patients, which may make the schizophrenic patient susceptible to the development of type 2 diabetes mellitus. However, further study of the interrelationships between *IGF2BP2* gene polymorphisms, expression of the *IGF2BP2* gene, and insulin secretion in schizophrenia as a risk factor for comorbid type 2 diabetes mellitus is warranted using a longitudinal design in first-episode and drug-naive patients with schizophrenia.

Both our genotype association and the mRNA expression studies have several limitations. First, although our sample size was large and provided high statistical power, the effect size shown as the allelic OR of 1.22 for both genders and 1.32 for men was modest. Second, we examined only 1 SNP in the *IGF2BP2* gene, and the coverage of the variants in the *IGF2BP2* gene is apparently insufficient. Future studies should investigate at least several coding variants in linkage disequilibrium with the SNP rs4402960, if any, and cover variation in other type 2 diabetes mellitus–related genes and adjust for multiple testing. Therefore, a neighboring variant or gene in linkage disequilibrium with the *IGF2BP2* rs4402960 polymorphism could also be functionally associated with schizophrenia. Examining nearby functional sites in or near the *IGF2BP2* rs4402960 polymorphism is warranted. Third, a replication trial in an independent sample is needed with a potentially larger sample size and from different ethnic populations in order to test for a false-positive association. However, we benefited from our homogeneous Northeast Chinese Han population that is robust against false-positive associations resulting from population stratification.[41] Fourth, all the patients in our mRNA expression study were taking antipsychotic medication at varying dosages, complicating any analysis of medication effects. Fifth, the identification of genetic loci associated with the co-occurrence of type 2 diabetes mellitus and schizophrenia is complicated by several factors, such as environmental and iatrogenic factors (eg, antipsychotics). However, we did not obtain information about antipsychotic treatments (typical or atypical, dosage or duration of treatment), body mass index, or other metabolic data, which are considered important risk factors for type 2 diabetes mellitus. Sixth, the gene expressions were conducted on leukocytes from peripheral blood samples; therefore, these data may not reflect brain changes. Seventh, a standard instrument for assessment of positive symptoms was not used, although the Andreasen scale for the negative symptoms was utilized. Eighth, we examined the mRNA expression of the *IGF2BP2* gene in separate samples. It should be interesting to examine whether the polymorphism rs4402960 was associated with the expression level of the *IGF2BP2* gene. Hence, future functional studies are needed to evaluate the biological mechanisms associated with the *IGF2BP2* gene variants and, further, to identify the SNPs of the *IGF2BP2* that are associated with variation in gene expression contributing to type 2 diabetes mellitus in schizophrenia. Ninth, although our finding a genetic association between schizophrenia and the polymorphism rs4402960 of *IGF2BP2* gene in male but not female schizophrenic patients is consistent with an increased risk of type 2 diabetes mellitus in male schizophrenic patients,[1–3,5] we did not find a significant gender difference in the *IGF2BP2* mRNA expression levels in schizophrenic patients. This inconsistency may reflect the smaller sample size in the expression study or some unknown mechanisms that deserve further investigation.

In summary, we showed that the *IGF2BP2* rs4402960 gene polymorphism, which is associated with type 2 diabetes mellitus, is associated with vulnerability to schizophrenia in a Han Chinese population, suggesting shared genetic risk variants. Furthermore, up-regulation of *IGF2BP2* gene may be involved in the pathophysiology of schizophrenia. However,

this finding remains preliminary because we do not have a replication sample for this association between schizophrenia and the rs4402960 polymorphism of the *IGF2BP2* gene. We cannot exclude the results as a false-positive until it is replicated in other independent larger samples with schizophrenia from different ethnicities.

Author affiliations: Research Center for Diabetic Complication and Department of Nephrology, the Second Hospital (Drs X. Zhang, Liu, You, Miao, and X. Y. Zhang); Research Center for Genomic Medicine and MH Radiobiology Research Unit, School of Public Health (Drs Hui, Wang, Sun, and Guan), Jilin University, Changchun, China; Department of Psychiatry, Chinese University of Hong Kong, Tai Po Hong Kong SAR, China (Dr Xiang); and the Menninger Department of Psychiatry and Behavioral Sciences, Baylor College of Medicine, and Michael E. DeBakey VA Medical Center, Houston, Texas (Drs Kosten and X. Y. Zhang).

Potential conflicts of interest: The authors have no conflicts of interest to disclose.

Funding/support: This study was funded by the National Natural Science Foundation of China (81171064); the Program for New Century Excellent Talents in University (NCET-10-0449); Jilin University Science Foundation for Outstanding Scholarship (201100002); the Stanley Medical Research Institute (03T-459 and 05T-726); the Department of Veterans Affairs, VISN 16; and the National Institutes of Health K05-DA0454, P50-DA18827, and U01-MH79639.

REFERENCES

1. Rouillon F, Sorbara F. Schizophrenia and diabetes: epidemiological data. *Eur Psychiatry*. 2005;20(suppl 4):S345–S348.
2. Smith M, Hopkins D, Peveler RC, et al. First- v second-generation antipsychotics and risk for diabetes in schizophrenia: systematic review and meta-analysis. *Br J Psychiatry*. 2008;192(6):406–411.
3. Lin PI, Shuldiner AR. Rethinking the genetic basis for comorbidity of schizophrenia and type 2 diabetes. *Schizophr Res*. 2010;123(2–3):234–243.
4. Chien IC, Hsu JH, Lin CH, et al. Prevalence of diabetes in patients with schizophrenia in Taiwan: a population-based National Health Insurance study. *Schizophr Res*. 2009;111(1–3):17–22.
5. Bresee LC, Majumdar SR, Patten SB, et al. Prevalence of cardiovascular risk factors and disease in people with schizophrenia: a population-based study. *Schizophr Res*. 2010;117(1):75–82.
6. Holt RI. Consensus development conference on antipsychotic drugs and obesity and diabetes: response to consensus statement. *Diabetes Care*. 2004; 27(8):2086–2087, author reply 2089–2090.
7. Henderson DC. Atypical antipsychotic-induced diabetes mellitus: how strong is the evidence? *CNS Drugs*. 2002;16(2):77–89.
8. Citrome LL, Holt RI, Zachry WM, et al. Risk of treatment-emergent diabetes mellitus in patients receiving antipsychotics. *Ann Pharmacother*. 2007;41(10): 1593–1603.
9. Nasrallah HA. Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles. *Mol Psychiatry*. 2008;13(1):27–35.
10. Gough SC, O'Donovan MC. Clustering of metabolic comorbidity in schizophrenia: a genetic contribution? *J Psychopharmacol*. 2005;19(suppl): 47–55.
11. Mukherjee S, Decina P, Bocola V, et al. Diabetes mellitus in schizophrenic patients. *Compr Psychiatry*. 1996;37(1):68–73.
12. Spelman LM, Walsh PI, Sharifi N, et al. Impaired glucose tolerance in first-episode drug-naïve patients with schizophrenia. *Diabet Med*. 2007;24(5): 481–485.
13. Ryan MC, Collins P, Thakore JH. Impaired fasting glucose tolerance in first-episode, drug-naive patients with schizophrenia. *Am J Psychiatry*. 2003; 160(2):284–289.
14. Marder SR, Essock SM, Miller AL, et al. Physical health monitoring of patients with schizophrenia. *Am J Psychiatry*. 2004;161(8):1334–1349.
15. Guest PC, Wang L, Harris LW, et al. Increased levels of circulating insulin-related peptides in first-onset, antipsychotic naïve schizophrenia patients. *Mol Psychiatry*. 2010;15(2):118–119.
16. Freeman H, Rodnick EH, Shakow D, et al. The carbohydrate tolerance of mentally disturbed soldiers. *Psychosom Med*. 1944;6(4):311–317.
17. Braceland FJ, Meduna LJ, Vaichulis JA. Delayed action of insulin in schizophrenia. *Am J Psychiatry*. 1945;102(1):108–110.
18. Alkelai A, Greenbaum L, Lupoli S, et al. Association of the type 2 diabetes mellitus susceptibility gene, TCF7L2, with schizophrenia in an Arab-Israeli family sample. *PLoS ONE*. 2012;7(1):e29228.
19. Hammer NA, Hansen TO, Byskov AG, et al. Expression of IGF-II mRNA-binding proteins (IMPs) in gonads and testicular cancer. *Reproduction*. 2005;130(2):203–212.
20. Christiansen J, Kolte AM, Hansen TO, et al. IGF2 mRNA-binding protein 2: biological function and putative role in type 2 diabetes. *J Mol Endocrinol*. 2009;43(5):187–195.
21. Zeggini E, Weedon MN, Lindgren CM, et al; Wellcome Trust Case Control Consortium (WTCCC). Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science*. 2007;316(5829): 1336–1341.
22. Jia H, Yu L, Jiang Z, et al. Association between IGF2BP2 rs4402960 polymorphism and risk of type 2 diabetes mellitus: a meta-analysis. *Arch Med Res*. 2011;42(5):361–367.
23. Wu J, Wu J, Zhou Y, et al. Quantitative assessment of the variation in IGF2BP2 gene and type 2 diabetes risk. *Acta Diabetol*. 2012;(49)Suppl:87–97.
24. Andreasen NC. *The Scale for the Assessment of Negative Symptoms (SANS)*. Iowa City, IA: University of Iowa; 1983.
25. Steve Rozen and Helen J. Skaletsky. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, 2000; 365–386.
26. Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21(2):263–265.
27. Dudbridge F. Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. *Hum Hered*. 2008;66(2):87–98.
28. Gauderman WJ. Sample size requirements for matched case-control studies of gene-environment interaction. *Stat Med*. 2002;21(1):35–50.
29. Saxena R, Voight BF, Lyssenko V, et al; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science*. 2007;316(5829):1331–1336.
30. Scott LJ, Mohlke KL, Bonnycastle LL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science*. 2007;316(5829):1341–1345.
31. Zhao Y, Ma YS, Fang Y, et al. IGF2BP2 genetic variation and type 2 diabetes: a global meta-analysis. *DNA Cell Biol*. 2012;31(5):713–720.
32. Mukherjee S, Schnur DB, Reddy R. Family history of type 2 diabetes in schizophrenic patients. *Lancet*. 1989;333(8636):495.
33. Holt RI, Bushe C, Citrome L. Diabetes and schizophrenia 2005: are we any closer to understanding the link? *J Psychopharmacol*. 2005;19(suppl):56–65.
34. Fernandez-Egea E, Miller B, Bernardo M, et al. Parental history of type 2 diabetes in patients with nonaffective psychosis. *Schizophr Res*. 2008;98(1–3): 302–306.
35. Hansen T, Ingason A, Djurovic S, et al. At-risk variant in TCF7L2 for type II diabetes increases risk of schizophrenia. *Biol Psychiatry*. 2011;70(1):59–63.
36. Irvin MR, Wiener HW, Perry RP, et al. Genetic risk factors for type 2 diabetes with pharmacologic intervention in African-American patients with schizophrenia or schizoaffective disorder. *Schizophr Res*. 2009;114(1–3):50–56.
37. Lorenz WF. Sugar tolerance in dementia praecox and other mental disorders. *Arch Neurol Psychiatry*. 1922;8(2):184–196.
38. Nielsen J, Christiansen J, Lykke-Andersen J, et al. A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. *Mol Cell Biol*. 1999;19(2):1262–1270.
39. Grarup N, Rose CS, Andersson EA, et al. Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. *Diabetes*. 2007;56(12):3105–3111.
40. Groenewoud MJ, Dekker JM, Fritsche A, et al. Variants of CDKAL1 and IGF2BP2 affect first-phase insulin secretion during hyperglycaemic clamps. *Diabetologia*. 2008;51(9):1659–1663.
41. Ewens WJ, Spielman RS. The transmission/disequilibrium test: history, subdivision, and admixture. *Am J Hum Genet*. 1995;57(2):455–464.