TABLE I. Genotypic and Allelic Distributions of Three SNPs of the *GRM3* Gene

Polymorphism	Area[a]	Group		Genotype count (frequency)						*P*	Allele count (frequency)				*P*	HWE *P*
rs274622				CC		CT		TT			C		T			
	WJ	Affected	n = 475	13	(0.03)	150	(0.32)	312	(0.66)		176	(0.19)	774	(0.81)		0.315
	WJ	Controls	n = 477	14	(0.03)	152	(0.32)	311	(0.65)	0.85	180	(0.19)	774	(0.81)	0.85	0.373
	EJ	Affected	n = 1439	37	(0.03)	403	(0.28)	999	(0.69)		477	(0.17)	2401	(0.83)		0.630
	EJ	Controls	n = 1438	35	(0.02)	390	(0.27)	1013	(0.70)	0.55	460	(0.16)	2416	(0.84)	0.55	0.726
	Total	Affected	n = 1914	50	(0.03)	553	(0.29)	1311	(0.68)		653	(0.17)	3175	(0.83)		0.357
		Controls	n = 1915	49	(0.03)	542	(0.28)	1324	(0.69)	0.68	640	(0.17)	3190	(0.83)	0.68	0.463
rs1468412				AA		AT		TT			A		T			
	WJ	Affected	n = 479	319	(0.67)	142	(0.30)	18	(0.04)		780	(0.81)	178	(0.19)		0.658
	WJ	Controls	n = 477	293	(0.61)	163	(0.34)	21	(0.04)	0.11	749	(0.79)	205	(0.21)	0.11	0.781
	EJ	Affected	n = 1436	924	(0.64)	457	(0.32)	55	(0.04)		2305	(0.80)	567	(0.20)		0.872
	EJ	Controls	n = 1432	935	(0.65)	445	(0.31)	52	(0.04)	0.58	2315	(0.81)	549	(0.19)	0.58	0.916
	Total	Affected	n = 1915	1243	(0.65)	599	(0.31)	73	(0.04)		3085	(0.81)	745	(0.19)		0.937
		Controls	n = 1909	1228	(0.64)	608	(0.32)	73	(0.04)	0.74	3064	(0.80)	754	(0.20)	0.74	0.834
rs2299225				GG		GT		TT			G		T			
	WJ	Affected	n = 479	8	(0.02)	83	(0.17)	388	(0.81)		99	(0.10)	859	(0.90)		0.155
	WJ	Controls	n = 477	10	(0.02)	105	(0.22)	362	(0.76)	0.07	125	(0.13)	829	(0.87)	0.06	0.466
	EJ	Affected	n = 1437	18	(0.01)	309	(0.22)	1110	(0.77)		345	(0.12)	2529	(0.88)		0.499
	EJ	Controls	n = 1433	22	(0.02)	310	(0.22)	1101	(0.77)	0.69	354	(0.12)	2512	(0.88)	0.69	0.973
	Total	Affected	n = 1916	26	(0.01)	392	(0.20)	1498	(0.78)		444	(0.12)	3388	(0.88)		0.951
		Controls	n = 1910	32	(0.02)	415	(0.22)	1463	(0.77)	0.20	479	(0.13)	3341	(0.87)	0.20	0.681

[a]Area where the subjects lived, WJ: western Japan (Okayama area); EJ: central to eastern Japan (Kanto, Niigata, Nagoya area) HWE: Hardy–Weinberg Equilibrium.

TABLE II. Estimated Haplotype Frequencies of the *GRM3* Gene

Haplotype	Schizophrenia frequency	Control frequency	*P*
rs274622-rs1468412-rs2299225			
T-A-T	0.65	0.65	0.81
C-A-T	0.16	0.15	0.59
T-T-G	0.11	0.12	0.23
T-T-T	0.07	0.07	0.19
rs274622-rs1468412			
T-A	0.65	0.65	0.81
T-T	0.18	0.18	0.93
C-A	0.16	0.15	0.52
C-T	0.01	0.01	0.44
rs1468412-rs2299225			
A-T	0.80	0.80	0.84
T-G	0.11	0.12	0.18
T-T	0.08	0.07	0.21

odds ratio = [OR] 1.92; 95% confidence interval [CI] = 1.18–3.12). Chen et al. [2005] reported that the C allele of the rs2299225 SNP in intron 3 of GRM3 was found more frequently in 752 Chinese patients with schizophrenia than in 752 control subjects ($P = 0.03$; OR = 1.44; 95% CI = 1.05–1.99). Egan et al. [2004] reported that the A allele of the rs6465084 SNP in intron 2 was overtransmitted to affected offspring in European-American families included in the Clinical Brain Disorders Branch "Sibling Study" ($P = 0.02$); however, this allele was undertransmitted to affected offspring in Caucasian families, who participated in the National Institute of Mental Health Genetics Initiative (NIMHGI; $P = 0.27$). These three papers also reported haplotype associations with schizophrenia. However, the SNPs used to construct haplotypes differed between the studies. Marti et al. [2002] reported that the T allele of rs2228595, a synonymous SNP in exon 3, was overrepresented in 265 German schizophrenia patients compared with that of 227 control subjects ($P = 0.002$). However, this association was not replicated in another study of 289 German patients and 163 control subjects ($P = 0.57$). Fallin et al. [2005] reported significant associations of 4-SNP haplotypes of GRM3 with bipolar disorder but not with schizophrenia in Ashkenazi Jewish case-parent trios. Tochigi et al. [2006] did not observe associations between 10 SNPs in GRM3 and schizophrenia in 402 Japanese patients with schizophrenia and 468 Japanese control subjects. Thus, the findings that support associations of genetic variations in GRM3 with schizophrenia are not compelling, although GRM3 is regarded as one of the most promising candidate genes [Harrison and Weinberger, 2005].

Genetic association studies are prone to spurious findings and type 1 errors, and therefore, replication studies are essential before genetic association is accepted. However, in replication studies, the sample size is critical because the association of SNPs with schizophrenia is thought to be weak. Studies without sufficient power to replicate a previously reported association are prone to type 2 errors. In the present study, we attempted to confirm associations of two SNPs in GRM3 reported to be associated with schizophrenia in Asian populations [Fujii et al., 2003; Chen et al., 2005]. In addition to these two SNPs, we genotyped one SNP, rs274622, that was reported to be associated with negative symptom improvement in Caucasian schizophrenia patients treated with olanzapine [Bishop et al., 2005].

MATERIALS AND METHODS

Subjects

All subjects were of Japanese descent and were recruited from the main island of Japan. A total of 1,916 unrelated patients with schizophrenia (mean age ± SD, 48.9 ± 14.5 years; 1058 men and 858 women) were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). Control subjects were 1,915 mentally healthy, unrelated subjects (mean age ± SD, 49.0 ± 14.3 years; 1,044 mens and 871 womens) with no self-reported family history of mental illness within second-degree relatives. The present study was approved by the Ethics Committees of the University of Tsukuba, Niigata University, Fujita Health University, Nagoya University, Okayama University, and Teikyo University. All participants provided written informed consent.

Genotyping

DNA was extracted from blood samples. We genotyped three SNP markers, rs274622 in the promoter region, and rs1468412 and rs2299225 in intron 3 of GRM3. The SNPs were genotyped by TaqMan assay. Predesigned TaqMan SNP genotyping assays, C_2293486_10 for rs274622, C_15752033_10 for rs1468412, and C_7586401_10 for rs2299225, were selected from the Applied Biosystems database (http://www.appliedbiosystems.com). The TaqMan reaction was performed in a final volume of 3 μl consisting of 2.5 ng genomic DNA and Universal Master Mix (Eurogentic, Seraing, Belgium). Genotyping was performed with an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA).

Statistical Analysis

Hardy–Weinberg equilibrium, linkage disequilibrium, and allelic/haplotype frequencies, as well as an association between an SNP or haplotype and schizophrenia, were evaluated with the Haploview software program (http://www.broad.mit.edu/mpg/haploview/). Permutation tests were also performed to calculate corrected P values for multiple testing with the Haploview software. Genotype-based association was tested with Cochran–Armitage test for trends. Statistical significance was accepted at $P < 0.05$.

Meta-analysis was performed with the Mantel–Haenszel method as a fixed-effect model test and the DerSimonian–Laird method as a random-effects model test. Heterogeneity among studies was tested with the χ^2 statistic obtained by adding the weighted squares of the deviation of each estimate from the pooled estimate. Publication bias was not examined because the number of reports was small. We estimated ORs by comparing patients with schizophrenia with control subjects in the same study and calculated ORs under the hypothesis that the A and G alleles of rs1468412 and rs2299225, respectively, were more susceptible to schizophrenia as shown in previous studies [Fujii et al., 2003; Chen et al., 2005].

TABLE III. Meta-Analysis of Five Case-Control Studies of GRM3 Polymorphisms and Schizophrenia

		The A allele of rs1468412					The G allele of rs2299225				
References	Population	Patients with schizophrenia (n)	Control (n)	OR	(95% CI)	*P*	Patients with schizophrenia (n)	Control (n)	OR	(95% CI)	*P*
Fujii et al. (2003)	Japanese	100	100	1.92	(1.18–3.12)	0.01					
Chen et al. (2005)	Chinese	735	733	0.95	(0.75–1.20)	0.22	751	750	1.44	(1.05–1.99)	0.03
Norton et al. (2005)	German	663	698	0.93	(0.79–1.10)	0.41					
Tochigi et al. (2006)	Japanese	402	457	0.89	(0.74–1.07)	0.65	401	463	1.20	(0.89–1.63)	0.23
Present Study	Japanese	1915	1909	1.02	(0.91–1.14)	0.74	1916	1910	0.91	(0.80–1.05)	0.2
Combined fixed-effects model (Asian)				1.00	(0.92–1.09)	1.00			1.01	(0.91–1.13)	0.81
Combined random-effects model (Asian)		3152	3199	1.03	(0.91–1.14)	0.61	3068	3123	1.13	(0.85–1.51)	0.52
Combined fixed-effects model (total)				0.99	(0.92–1.06)	0.87					
Combined random-effects model (total)		3815	3897	1.00	(0.87–1.14)	1.00					

RESULTS

Genotypic/allelic distributions of the three GRM3 SNPs among the subject groups are shown in Table I. Distributions of these SNPs did not differ significantly from Hardy–Weinberg equilibrium. No significant genotype/allelic association with schizophrenia was detected for rs274622 (genotypic $P = 0.68$/allelic $P = 0.68$), rs1468412 (genotypic $P = 0.74$/allelic $P = 0.74$), or rs2299225 (genotypic $P = 0.20$/allelic $P = 0.20$). Populations in east and west Japan have slightly different cultural backgrounds. Therefore, we divided our subjects into those who lived in eastern and western Japan. No significant association was observed (Table I). These SNPs were in linkage disequilibrium with each other in both the control and patient groups as reported by Tochigi et al. [2006] ($D' = 0.60$ and $r^2 = 0.02$ between rs274622 and rs1468412; $D' = 0.98$ and $r^2 = 0.54$ between rs1468412 and rs2299225; $D' = 0.78$ and $r^2 = 0.02$ between rs274622 and rs2299225, respectively, in the total subjects). Haplotype frequencies did not differ significantly between the schizophrenia and control groups (Table II).

The total number of subjects included in the meta-analysis was 3,815 patients with schizophrenia and 3,897 control subjects for rs1468412 and 3,068 patients and 3,123 control subjects for rs2299225 (Table III). Overall, the combined OR of the fixed-effects model for schizophrenia with rs1468412 was 0.99 (95% CI = 0.92–1.06; $P = 0.87$; heterogeneity $P = 0.06$), and that of rs2299225 was 1.01 (95% CI = 0.91–1.05; $P = 0.67$; heterogeneity $P = 0.02$). Meta-analysis limited to Asian studies also yielded no significant results (Table III).

DISCUSSION

We attempted to replicate genetic associations between rs1468412 and rs2299225 that were previously reported to be associated with schizophrenia in a Japanese population [Fujii et al., 2003] and a Chinese population [Chen et al., 2005], respectively. We were not successful in replicating either association. As shown in Table III, Fujii et al. [2003] reported the OR for schizophrenia with the A allele of rs1468412 was 1.92 (95% CI = 1.18–3.12), whereas, in the present study, the OR was 1.02 (95% CI = 0.91–1.14). The power of detection for the OR of 1.18 (the lower end of the 95% CI for OR reported by Fujii et al. [2003]), was >0.9 in the present study. Moreover, the result of the meta-analysis did not support the association of rs1468412 with schizophrenia. The meta-analysis of the present study did not include transmission disequilibrium test (TDT) data for rs1468412 which were reported by Egan et al. [2004]. However, their study did not detect a significant association of the SNP with schizophrenia.

Chen et al. [2005] reported that the OR for association of the G allele of rs2299225 with schizophrenia was 1.44 (95% CI = 1.05–1.99), whereas the OR in the present study was 0.91 (95% CI = 0.8–1.05). The 95% CIs of the ORs of the study reported by Chen et al. [2005] and the present study did not overlap. The meta-analysis in the present study did not support the association of rs2299225 with schizophrenia. Thus, the present study provides further evidence that neither SNP in GRM3 is associated with schizophrenia.

In the present study, significant haplotype associations with schizophrenia were not detected. Chen et al. [2005] reported that haplotype of C (rs2237562)—T (rs1468412)—C (rs2299225) was detected more frequently in patients with schizophrenia than in control subjects ($P = 0.008$). Although we did not genotype rs2237562, the rs1468412 and rs2299225 SNPs can determine the haplotype due to linkage disequilibrium. We observed no difference in frequencies of haplotypes constructed by rs1468412 and rs2299225 between patients with schizophrenia and controls in the present study (Table II). Fujii et al. [2003] reported significant associations of seven 2-SNP haplotypes and

five 3-SNP haplotypes, including the haplotype constructed with rs274622 and rs1468412 ($P = 0.002$), with schizophrenia. The present study, however, did not replicate the rs274622 and rs1468412 haplotype associations (Table II).

In the present study, we examined only three SNPs. Therefore, the present study does not exclude the possibility of associations between other variations in GRM3 and schizophrenia. Although the GRM3 single marker and haplotype associations with schizophrenia have not been replicated so far in other populations, further studies exploring associations of genetic variations not in linkage disequilibrium with the GRM3 SNPs or haplotypes examined thus far with schizophrenia are necessary. Furthermore, epistatic interactions between variations in GRM3 and those in other genes remain mostly to be investigated. A marginal significant interaction between polymorphisms in GRM3 and catechol-*O*-methyltransferase (COMT) Val158Met has been reported recently [Nicodemus et al., 2007].

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RESEARCH ARTICLE

Variants of dopamine and serotonin candidate genes as predictors of response to risperidone treatment in first-episode schizophrenia

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Aims: Abnormalities in dopaminergic and serotonergic transmission systems are thought to be involved in the pathophysiology of schizophrenia and the mechanisms underlying the therapeutic effects of antipsychotics. We conducted a pharmacogenetic study to evaluate whether variants in dopamine-related genes (*DRD1–DRD5*, *AKT1* and *GSK3β*) and serotonin receptor genes (*HTR1A*, *HTR1B*, *HTR1D*, *HTR2A*, *HTR2C*, *HTR6* and *HTR7*) can be used to predict the efficacy of risperidone treatment for schizophrenia.
Materials & methods: A total of 120 first-episode neuroleptic-naive schizophrenia patients were treated with risperidone monotherapy for 8 weeks and clinical symptoms were evaluated by the Positive and Negative Syndrome Scale. **Results:** Among the 30 variants that we examined, two SNPs in *DRD2* (-241A>G [rs1799978] and TaqIA [rs1800497]) and two SNPs in *AKT1* (*AKT1*-SNP1 [rs3803300] and *AKT1*-SNP5 [rs2494732]) were significant predictors of treatment response to risperidone. **Conclusion:** These data suggest that the SNPs in *DRD2* and *AKT1* may influence the treatment response to risperidone in schizophrenia patients.

Keywords: dopamine, pharmacogenetics, risperidone, schizophrenia, serotonin

Schizophrenia is a severe psychiatric disorder with a lifetime risk of 1%. Its pathophysiology is unknown, as are the mechanisms underling therapeutic response to treatment. Similarly, the reasons for variable individual responses to treatment are not known and, at present, the choice of particular antipsychotic treatments for individual patients is effectively a trial and error process. However, since genetic factors contribute to treatment response [1], pharmacogenetic approaches offer at least the potential for predicting treatment response at an individual level

With respect to the use of antipsychotic medication in schizophrenia, pharmacogenetic research has focused on dopamine- and serotonin-related genes. In particular, several groups [1–13] have targeted genes encoding the dopamine D2 receptor (*DRD2*: -141Ins/del [rs1799732] and TaqIA polymorphisms [rs1800497; although TaqIA is now known to be located in another proximal gene: X-kinase or ANKK1]), the D3 receptor (*DRD3*: Ser9Gly [rs6280]), the D4 receptor (*DRD4*: 48-bp repeat in exon III), and serotonin 5-HT2A (*HTR2A*: T102C [rs6313]) and 5-HT1A receptors (*HTR1A*: -1019C/G [rs6295]). However, the results of these studies have been discrepant [1,2]. This may be because variation in each individual gene may have weak effects [14]. It has been postulated that interaction between variants in dopaminergic and serotonergic systems may be of greater magnitude in predicting responses to treatment of schizophrenia [15]. The candidate polymorphisms in such genes should be integrated for precise analysis; therefore, we can evaluate the individual gene effects and gene–gene interaction to antipsychotic treatment.

Many candidate genes have been proposed to be of pharmacogenetic relevance to antipsychotic treatment; compared with typical antipsychotics, second-generation antipsychotics, including risperidone, have lower (to similar) affinity for the D2 receptor and a higher degree of occupancy at four other dopamine receptors (D1, D3, D4 and D5) and at some serotonin receptors (5-HT1A, 1B, 1D, 2A, 6 and 7, and other receptors) [16]. In addition, variation in signaling cascades downstream of D2 receptor blockage may be associated with treatment of schizophrenia [17]. Among these, the AKT/glycogen synthase kinase 3 (GSK3) signaling cascade is a particularly attractive candidate. First, antipsychotics, including risperidone, alter the expression level of GSK3 protein in the rat medial prefrontal cortex and striatum [18]. Second, increased phosphorylation of AKT1 and GSK3β have been reported in mice exposed to haloperidol [19]. Third, in a study of dopamine transporter knockout and wild-type mice, AKT1–GSK3β signaling cascades partially mediated DA-dependent behaviors in response to manipulation by exposure to lithium and amphetamine [20]. Lastly, genetic aspects of susceptibility to schizophrenia and antipsychotic response may be closely associated [21]. This is of

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relevance to AKT1–GSK3β signaling, since several case–control and family-based association studies have provided some evidence for association between *AKT1* and schizophrenia [19,22–26], although the findings are not universal [27–30].

The aim of this study was to conduct a pharmacogenetic study of risperidone response and variants in genes encoding dopamine and serotonin receptors (*D1–D5, HTR1A, HTR1B, HTR1D, HTR2A, HTR2C, HTR6* and *HTR7*), *AKT1* and *GSK3β*. In a small sample, we previously reported that diplotypes at *DRD2* were associated with clinical performance after risperidone treatment, although no association was found between risperidone response and gene variants in *5-HT2A* and *COMT* [12]. In this study, we expanded the sample size of first-episode neuroleptic-naive samples (from 31 to 120 patients) and controlled for nongenetic factors such as clinical charactistics and environmental variables (gender, age, duration of untreated psychosis [DUP] and baseline Positive and Negative Syndrome Scale [PANSS] total score) by multiple linear regression analysis.

Materials & methods

Subjects & collection of clinical data

In total, 131 first-episode, neuroleptic-naive schizophrenic patients were included in this open-label pharmacogenetic study. For *DRD2* and *HTR2A*, 31 patients were the same as those included in our previous report [12]. We excluded ten patients whose DUP was longer than 5 years in accordance with another study [31]. Genotypes could not be determined in one subject, and this patient was also excluded. Consequently, 120 patients were analyzed.

Patients were entered into the study if they met diagnostic and statistical manual of mental disorders (DSM)-IV-TR criteria for schizophrenia, were physically healthy and had all laboratory parameters within normal limits, or if they had neither current nor past DSM-IV-TR diagnosis of mood disorders or substance abuse. Consensus diagnoses were made by at least two experienced psychiatrists on the basis of unstructured interviews with patients and families and review of medical records. DUP was defined as the period from the onset of psychotic symptoms to that of first antipsychotic exposure.

Subjects received risperidone monotherapy (starting dosage: 0.5–4 mg/day; mean starting dosage: 2.5 mg) and dosage was adjusted in accordance with patients' symptoms by trained psychiatrists (1–8 mg/day; mean dosage: 3.4 mg at 8 weeks) for 8 weeks. Patients with insomnia were prescribed brotizolam 0.25 or 0.5 mg at bedtime. No other psychotropic drugs were permitted.

Clinical symptoms were evaluated at the first visit and after 8 weeks of treatment by the use of the PANSS. Evaluations were carried out by trained psychiatrists and a psychologist (inter-rater reliability: intraclass correlation coefficient [ICC's] = 0.90 [Yamanouchi & Iwata, Unpublished Data]).

The clinical characteristics of subjects that were used as potential covariates in the stepwise linear regression analysis were selected from a previous paper [32]: gender (58 male, 62 female), age (31.2 ± 8.7 years), DUP (13.7 ± 11.4 months) and baseline PANSS total score (79.1 ± 20.5).

All patients were unrelated and were ethnically Japanese. After explanation of the study, written informed consent was obtained from each subject. This study was approved by the Ethics Committee at Fujita Health University, University of Occupational and Environmental Health and Nagoya University Graduate School of Medicine.

Variant selection & genotyping

In total, 30 variants were selected from previous studies (**Table 1**). We specifically targeted potential functional polymorphisms and those which were previously associated with treatment response or with schizophrenia itself. These include: four SNPs for *DRD1* [33]; three variants for *DRD2* [12,34]; two SNPs for *DRD3* [35]; four variants for *DRD4* [36,37]; one SNP for *DRD5* [38]; five SNPs for *AKT1* [22]; two SNPs for *GSK3B* [39]; one SNP for *HTR1A* [40]; one SNP for *HTR1B* [41]; one SNP for *HTR1D* [42]; one SNP for *HTR2A* [12]; two SNPs for *HTR2C* [43,44]; one SNP for *HTR6* [10]; and two SNPs for *HTR7* [45]. Genotyping methods can be seen in **Table 1** and primer sequences are available on request.

In *DRD1*, -1251*HaeIII* was not polymorphic while the three other SNPs (-800HaeIII, -48DdeI and +1403Bsp1286I) were in absolute linkage disequilibrium (LD; $r^2 = 1$) in our sample. Thus, we included only -800HaeIII in *DRD1* to the following regression analysis. At *DRD3*, -205G>A and Ser9Gly were similarly in LD ($r^2 = 1$), so we analyzed only Ser9Gly (**Table 1**). For the 48-bp repeat in exon III of *DRD4*, the allele frequencies of the variant differ considerably between populations, and the seven-repeat allele is quite rare in the Japanese population. Therefore, alleles with five or more repeats were grouped with the long L allele in accordance with another study [36]. For

Table 1. Distribution of genotypes and direct association between PANSS improvement and genotypes.

Gene symbol	SNP ID	Methods	n	Genotype			p-value*
				M/M	*M/m*	*m/m*	
DRD1‡	-1251HaeIII (G>C)	PCR-RFLP	120	120	0	0	NA
	-800HaeIII (C>T)	PCR-RFLP	120	99	21	0	0.686
DRD2	-241A>G	PCR-RFLP	120	96	24	0	0.447
	-141 Ins/Del (Ins>Del)	PCR-RFLP	120	90	30	0	0.435
	TaqIA (A2>A1)	PCR-RFLP	120	54	54	12	0.0239§
DRD3¶	Ser9Gly (Ser>Gly)	PCR-RFLP	120	60	55	5	0.989
DRD4	120 bp duplication (L>S)	PCR-RFLP	120	73	44	3	0.403
	-616G>C	Direct sequencing	120	56	57	7	0.193
	-521T>C	Direct sequencing	120	38	66	16	0.925
	48bp repeat in exon III (S>L)#	PCR	120	114	6	0	0.969
DRD5	1481C>T	PCR-RFLP	120	62	46	12	0.456
AKT1	SNP1 rs3803300 (G>A)	PCR-RFLP	120	34	56	30	0.102
	SNP2 rs1130214 (G>T)	PCR-RFLP	120	71	45	4	0.949
	SNP3 rs3730358 (C>T)	PCR-RFLP	120	89	31	0	0.676
	SNP4 rs2498799 (A>G)	PCR-RFLP	120	32	60	28	0.210
	SNP5 rs2494732 (C>T)	PCR-RFLP	120	65	44	11	0.0286§
GSK3B	SNP6 rs1574154 (C>T)	PCR-RFLP	120	37	56	27	0.525
	SNP8 rs2037547 (C>T)	PCR-RFLP	120	106	14	0	0.844
HTR1A	-1019C>G	TaqMan®	120	73	45	2	0.799
HTR1B	861G>C	PCR-RFLP	120	25	73	22	0.151
HTR1D	rs674386	TaqMan®	120	64	50	6	0.597
HTR2A	102T>C	PCR-RFLP	120	31	58	31	0.948
HTR2C	-759C>T	TaqMan®	120	105	7	8**	0.315
	-697C>G	TaqMan®	120	104	8	8**	0.222
HTR6	267C>T	PCR-RFLP	120	60	56	4	0.580
HTR7	SNP2 rs3808932	Primer extension	120	74	33	13	0.535
	SNP5 rs12412496	PCR-RFLP	120	60	40	20	0.0437§

p-value for direct association (analysis of variance).

‡*-48DdeI and +1403BspI1286I were in absolute LD.*

§*Significant p-values.*

¶*-205G>C was in absolute LD.*

#*Five patients had 2/2 repeat, 25 patients had 4/2 repeat, 84 patients had 4/4 repeat, five patients had 4/5 repeat, one patient had 4/7 repeat.*

**Hemizygotes.*

LD: Linkage disequilibrium; M: Major allele; m: Minor allele; NA: Not analyzed; PANSS: Positive and Negative Syndrome Scale.

the two SNPs (-759C>T and -697C>G) in *HTR2C* that are located on the X chromosome, we applied a dominant genetic model to the following regression analysis: wild-type homozygote and the combined group of heterozygotes and mutant homozygotes (however, there is no sample with mutant homozygotes in our sample: eight samples in men have hemizygotes both of -759C>T and -697C>G. Seven and eight samples in women have heterozygotes of -759C>T and -697C>G, respectively).

Statistical analysis

All SNPs were tested for deviation from the Hardy–Weinberg equilibrium using an exact test (SAS/Genetics, release 8.2, SAS Institute Inc., Tokyo, Japan).

To check first if there was evidence for association between PANSS improvement and genotype, one-way analysis of variance (ANOVA) was applied (JMP, 6.J, SAS Institute Inc.). We next performed a stepwise backward selection procedure with a p-value threshold of 0.10 for excluding

covariates. In those analyses, the dependent variable was improvement rate in total scores of PANSS (calculated as shown below) and the independent variables included genotype, gender, age, DUP and baseline PANSS total score (JMP, 6.J).

$$\text{Improvement rate} = \frac{(\text{PANSS at week 0}) - (\text{PANSS at week 8})}{\text{PANSS at week 0}}$$

For the baseline PANSS score, we expected that total symptoms, positive symptoms, negative symptoms and general psychopathology would be correlated. Therefore, we initially calculated the correlation by Spearman's rank correlation test. We also selected total PANSS score as the covariate due to its generality (all correlations between the total PANSS score and the other subscores showed significance, but other combinations were not always significant [data not shown]).

When the significant variables were obtained, the adjusted means of improvement rate in PANSS score for each genotype were estimated by the method of least squares, and the protected least square difference test was used to compare individual groups. The significance level for all statistical tests was set at a p-value of less than 0.05.

Results

The distributions of all SNPs were within the values expected from Hardy–Weinberg equilibrium.

The improvement in total PANSS scores followed a normal distribution (Shapillo–Wilk test: $W = 0.991$; $p = 0.629$). Using one-way ANOVA, we found that three polymorphisms (TaqIA, *AKT1*-SNP5 and *HTR7*-SNP5) were nominally associated with PANSS improvement (Table 1). By contrast, the stepwise regression analysis suggested that two SNPs in *DRD2* (-241A>G polymorphism: $p = 0.031$; TaqIA polymorphism: $p = 0.0075$) and two SNPs in *AKT1* (*AKT1*-SNP1: $p = 0.018$; *AKT1*-SNP5: $p = 0.02$) were significant contributors (Table 2). Considering clinical background, only the baseline PANSS total score was a significant contributor ($p = 0.0058$) to predicting response (worse scores at 0 week showed better response). We found no difference in diagnosis subtypes between genotype groups for the four SNPs that were found to be predictors for treatment response (data not shown).

To evaluate the quantitative risk of predictor genotypes, the adjusted improvement rate among each genotype was compared by *t*-statistic. The patients with the A/A genotype in -241A>G, the A1/A1 genotype in TaqIA or the T/T genotype in AKT1-SNP5 showed significantly better improvement in total PANSS score than those without the above genotypes in each SNP. For AKT1-SNP1, individuals with the G/A genotype showed a significantly better improvement than patients with the A/A genotype, and a nearly significant improvement compared with those with the G/G genotype ($p = 0.0697$) (Figure 1).

Discussion

In this study, we found that SNPs in *DRD2* and *AKT1* (-241A>G [rs1799978] and TaqIA [rs1800497] in *DRD2*, and *AKT1*-SNP1 [rs3803300] and *AKT1*-SNP5 [rs2494732] in *AKT1*) were significant predictors of the improvement in total PANSS score after risperidone treatment.

However, we found a number of disagreements between the ANOVA and the regression analyses (-241A>G, *AKT1*-SNP1 and *HTR7*-SNP5 in *HTR7* [rs12412496]), although two findings, TaqIA and *AKT1*-SNP5 were consistent between the two analyses. It is not surprising, and we hypothesize that this is derived from the effects of gene–gene or gene–environmental interaction, since multiple linear regression analysis can reflect or adjust the above effects.

Our data support a recent report regarding an association between -241A>G in *DRD2* and treatment response for schizophrenia [11], but do not support previous significant associations between other polymorphisms in dopamine- and serotonin-related genes [1]; in the Chinese population, the 'A' allele of the -241A>G polymorphism is the predictor allele of better treatment response to risperidone [11]. Our data replicated this finding, suggesting that it might be a true predictor for risperidone treatment in Asian populations in general. However, we need further replications for clinical use. In another study, the authors found that the 'G' allele (and -141Ins/Ins homozygote) is associated with a faster response time to antipsychotic treatment [9], but we cannot directly compare our present results with this finding, as our study did not contain multiple longitudinal assessment points.

Our previous pharmacogenetic study suggested that diplotypes in *DRD2* were associated with clinical response: compared with patients with the Ins-A2/Ins-A2 diplotype, PANSS total scores of patients with Ins-A2/Del-A1 showed 40% greater improvement [12]. In the present study, we decided not to use this type of analysis

Table 2. Genotypes and other clinical backgrounds associated with improvement rate in PANSS total score by a stepwise backward selection procedure.

Variable	p-value
Baseline PANSS total score (one-point increments)	0.0058
DRD2 -241A>G	0.0311
DRD2 TaqIA	0.0075
AKT1-SNP1 rs3803300	0.0183
AKT1-SNP5 rs2494732	0.0201

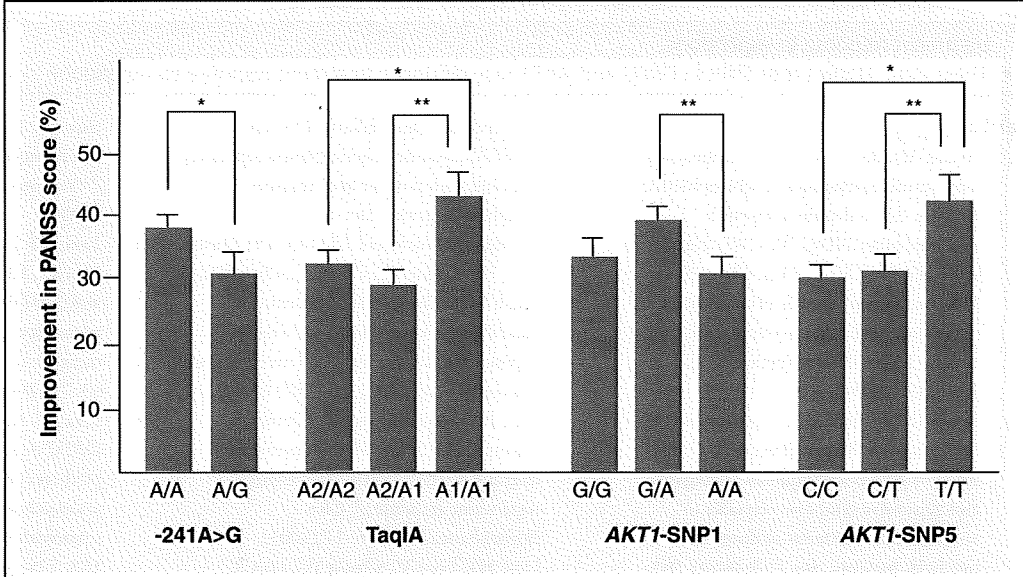
r^2: 0.25.
PANSS: Positive and Negative Syndrome Scale.

as a primary method because of weak LD between these variants (D′: 0.38; r^2: 0.045), but here we note that we undertook such an analysis but we could not confirm our previous findings (data not shown). The reasons for the discrepancies may derive from the background of the target samples; in our previous study, the subjects in the 'New' and 'Switched' groups were combined in order to increase the sample size, which might have caused a heterogeneous sample. By contrast, the present study included only first-episode, neuroleptic-naive subjects, resulting in pure phenotypes. In addition, the larger sample size in this study provided more statistical power than that in our previous study. In the previous report, TaqIA did not reach significance as a predictor (p = 0.08 [Yamanouchi & Iwata, Unpublished Data]), which might have been due to a type II error. However, a very likely explanation is that our previous positive report represented a type I error.

Concerning *AKT1*, it is an interesting finding that the identical variant, SNP5, may be a schizophrenia-susceptibility SNP [22] and a predictor SNP for risperidone treatment in schizophrenic patients. A recent pharmacogenetic study [25] showed no relationship between any of the tested SNPs in *AKT1* and therapeutic response to first- or second-generation antipsychotics. However, differences in LD among populations may also be responsible for such inconsistent results.

Emamian and colleagues showed that certain haplotypes (combination of SNP2 and 3) influenced the expression level of *AKT1* [19], but whether SNP1 or SNP5 are associated with function is unclear. To examine this, we developed a real-time reverse transcriptase PCR assay using lymphocyte-derived cell lines (25 subjects with schizophrenia and 25 age-matched healthy controls) to measure the expression of *AKT1* and *DRD2*. However, we found no evidence for association between any genotype and expression phenotype (data not shown). Since *AKT1* is an important intermediary between the D2 receptor and GSK3 proteins [19,20], *AKT1*-SNP5 and *AKT1*-SNP1 and/or SNPs in *DRD2* (-241A>G and TaqIA) may influence several signaling cascades in a gene–gene interaction manner.

Figure 1. Adjusted mean improvement rate in total PANSS score stratified by genotype in possible predictor SNPs.



Data shown are the mean ± SE. *p < 0.05; **p < 0.01.
PANSS: Positive and Negative Syndrome Scale.

Our study has a number of limitations. First, since we did not correct the p-values for multiple testing and sample size is relatively small, the results must be considered hypothesis generating and preliminary, and our nominally significant data need to be confirmed by independent samples. Second, we selected the patients who had completed 8 weeks of treatment, which might yield a false positive due to sampling bias as an open-label study. Third, we did not perform a comprehensive evaluation of any of the genes, for example, through a systematic LD-based study (e.g., tagging SNPs). Last, we did not detect the functional effect of these significant polymorphisms or did not include the fine mutation scan to search the actual causal variants. Therefore, the relationship between *DRD2* and *AKT1* polymorphisms and their functional relevance needs further study.

Conclusion & future perspective

We found predictor SNPs in *DRD2* and *AKT1* for risperidone treatment in schizophrenia patients. Further studies will be required for conslusive results, however, this evidence may lead to an improvement of response prediction and/or treatment selection for antipsychotics.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved

Executive summary

Risperidone response & dopamine- & serotonin-related genes

- In total, 120 first-episode neuroleptic-naive schizophrenic patients were examined to see if variants in dopamine-related genes (*DRD1–DRD5*, *AKT1* and *GSK3β*) and serotonin receptor genes (*HTR1A*, *HTR1B*, *HTR1D*, *HTR2A*, *HTR2C*, *HTR6* and *HTR7*) predicted the efficacy of risperidone treatment for schizophrenia.
- To reflect the gene–gene and gene–environmental interactions, multiple linear regression analysis was applied.

SNPs in DRD2 & AKT1 *were associated with risperidone response*

- Two SNPs in *DRD2* (-241A>G and TaqIA) and two SNPs in *AKT1* (*AKT1*-SNP1 and *AKT1*-SNP5) were significant predictors of treatment response to risperidone.
- These data suggest that SNPs in *DRD2* and *AKT1* may influence treatment response to risperidone in schizophrenia patients.

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IMMEDIATE COMMUNICATION

Analysis of 10 independent samples provides evidence for association between schizophrenia and a SNP flanking fibroblast growth factor receptor 2

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We and others have previously reported linkage to schizophrenia on chromosome 10q25–q26 but, to date, a susceptibility gene in the region has not been identified. We examined data from 3606 single-nucleotide polymorphisms (SNPs) mapping to 10q25–q26 that had been typed in a genome-wide association study (GWAS) of schizophrenia (479 UK cases/2937 controls). SNPs with $P<0.01$ ($n=40$) were genotyped in an additional 163 UK cases and those markers that remained nominally significant at $P<0.01$ ($n=22$) were genotyped in replication samples from Ireland, Germany and Bulgaria consisting of a total of 1664 cases with schizophrenia and 3541 controls. Only one SNP, rs17101921, was nominally significant after meta-analyses across the replication samples and this was genotyped in an additional six samples from the United States/Australia, Germany, China, Japan, Israel and Sweden ($n=5142$ cases/6561 controls). Across all replication samples, the allele at rs17101921 that was associated in the GWAS showed evidence for association independent of the original data (OR 1.17 (95% CI 1.06–1.29), $P=0.0009$). The SNP maps 85 kb from the nearest gene encoding fibroblast growth factor receptor 2 (*FGFR2*) making this a potential susceptibility gene for schizophrenia.
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Keywords: FGFR2; schizophrenia; genome-wide association study

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Introduction

Schizophrenia is a severe and debilitating psychiatric disorder with a lifetime risk of approximately 1%. Symptoms include hallucinations and delusions,

disorganized behavior, reduced drive and altered emotional reactivity.[1] Pathophysiology is largely unknown, but genes are known to make a major contribution to risk and heritability is greater than 0.8.[2] Epidemiological studies suggest that disease predisposition is likely to be the result of multiple genes of moderate to small effect,[3] a conclusion that is supported by meta-analysis of linkage studies,[4] by positional cloning studies,[5–8] and now by genome-wide association analysis.[9] Molecular genetic studies also suggest that a proportion of cases are associated with submicroscopic chromosomal abnormalities often referred to as copy number variations.[10–14]

We previously undertook a genome-wide linkage study of schizophrenia based on 353 affected sibling pairs from the United Kingdom, Sweden and the United States. Our strongest finding was a genome-wide significant logarithm of odds (LOD) score of 3.87 at chromosome 10q25.3–q26.3 (116–135 Mb).[15] Six other independent studies of schizophrenia or bipolar disorder (BD) have also reported linkage to chromosome 10q25–q26,[16–21] (Figure 1). Levinson *et al.*,[22] reported a genome scan of schizophrenia using 48 pedigrees from Australia and the United States. One of the most significant findings was at D10s1239 (103 Mb), with a nonparametric linkage score of 2.02. Mowry *et al.*[16] reported a follow-up of the more promising regions from that study based upon greater marker density and an extended sample. They obtained supportive evidence for linkage to 10q, although not at genome-wide significant levels. Lerer *et al.*,[19] also reported data from a genome scan of schizophrenia in 21 large families (155 affected individuals) from Israel. They found suggestive evidence for linkage to a region spanning D10s543 (112 Mb) to D10s587 (125 Mb). More recently, genome-wide linkage analysis of 409 schizophrenia pedigrees of European ancestry and African-American ancestry,[21] showed suggestive evidence for linkage at 10q25.3–q26.3, with a LOD-1 region of 116–134 Mb.

There are also three reports of linkage to this region in BD, a disorder that may share some genetic liability with schizophrenia.[23] First, Kelsoe *et al.*,[17] found suggestive evidence for linkage from D10s1237 (116 Mb) to D10s217 (129 Mb) in 20 multiplex families. Second, in a genome scan of 75 families from Germany, Israel and Italy, Cichon *et al.*,[18] reported suggestive evidence for linkage from D10s1757 (122 Mb) to D10s217 (129 Mb). Third, Liu *et al.*,[20] identified the region flanked by D10s1248 (131 Mb) to D10s169 (132 Mb) as suggestive for linkage to BD in 57 extended pedigrees from the United States and Israel. Finally, the study of Ewald *et al.*,[24] also gives supportive evidence for both BD and schizophrenia loci at 10q25.3–q26.3. Using haplotype-sharing methods in 15 BD patients and 10 with schizophrenia compared to control subjects from a relatively isolated population in the Faroe Islands, Ewald *et al.*,[24] showed evidence for association of the region flanked by D10s1230 (123 Mb) and D10s2322 (126 Mb) to both schizophrenia and BD.

Despite these reports of significant and suggestive linkage, no schizophrenia susceptibility genes mapping within this region have yet been identified. Aiming to do so, we have now examined the data we obtained from a genome-wide association (GWAS) study of schizophrenia[9] for evidence for association to the broad linkage region on 10q. Single-nucleotide polymorphisms (SNPs) with evidence for association surpassing nominal thresholds in our sample were genotyped in a series of samples totaling up to nine independent case–control series. Our data provide evidence across 10 case–control series for association between a SNP 85 kb from the fibroblast growth factor receptor 2 (*FGFR2*) locus.

Materials and methods

Overview of study design

SNPs from our GWAS sample (479 cases, 2937 controls) mapping within our linkage region (chr10 bases 115 673 689–135 374 737; UCSC March 2006) were inspected for evidence for association. Markers for which there was evidence for association at $P \leqslant 0.01$were genotyped in an additional 163 UK cases and, after confirming genotyping consistency with the GWA data, the data were merged giving a total of 642 cases and 2937 controls. For markers remaining significant at $P < 0.01$ in this extended UK sample, we sought independent replication in a combined analysis (1664 cases, 3541 controls) of 3 additional schizophrenia case–control series from Ireland (295 cases, 983 controls), Germany (Munich) (758 cases,

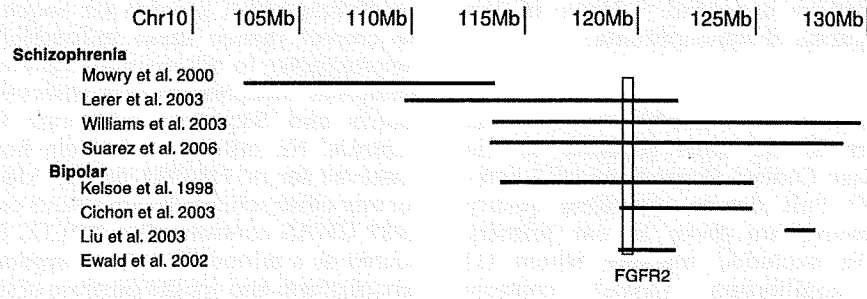

Figure 1 Linkage findings at chr10q25–q26. Mb; megabase.

1897 controls) and Bulgaria (611 cases, 661 controls). Markers that showed significant association at $P \leqslant 0.05$ to the same allele that was associated in the UK sample were genotyped in 6 more case–control series (combined $n = 6806$ cases, $n = 10102$ controls) from Germany (Bonn) (735 cases, 1036 controls), United States/Australia (1744 cases, 1938 controls), China (1034 cases, 1034 controls), Japan (748 cases, 831 controls), Israel (741 cases, 1517controls) and Sweden (140 cases, 205 controls). Finally, we also combined in our analysis the data from The Wellcome Trust Case Control Consortium (WTCCC) study of BD.[25]

Subjects

Full details of the UK and replication samples from the United States/Australia, Germany, Ireland, Bulgaria, Israel, China and Japan are previously described.[9] All patients from the United Kingdom, Ireland, Germany, Bulgaria, China, Japan and the Ashkenazi Jewish samples had a Diagnostic and Statistical Manual of Mental Disorders (DSMIV) diagnosis of schizophrenia. Patients from the US/Australian sample all had a consensus lifetime best-estimate diagnosis of DSMIV schizophrenia (90%) or schizoaffective disorder (10%). All patients and controls gave written and informed consent to participate.

Swedish sample

The Swedish sample consisted of 140 unrelated cases (86 men and 54 women) 91 of whom were included in our linkage sample.[15] Cases were ascertained through the Mental Health Inpatient Register and the Swedish Second Generation Register. Patients who had a register diagnosis of 'ICD-10 schizophrenia' were included. All had at least 1 affected first-degree relative, were white and were born in Sweden. All gave written, informed consent, as approved by the institutional review board of the Karolinska Institute. The control sample consisted of 205 subjects (130 men, 75 women), with a mean age of 40.2 (s.d. 10.2) years, age range 19–86 years. The majority had previously served as healthy controls subjects in biological psychiatric research at the Karolinska Institute and were reassessed for lifetime psychiatric diagnosis using structured interviews, whereas a minority ($n = 13$) was drawn from a representative sample of the population in Stockholm County and assessed as previously described.[26] None of the controls had a diagnosis of schizophrenia.

Genotyping

The GWAS sample of 479 cases and 2937 controls was genotyped as part of the same pipeline as the Wellcome Trust Case Control Consortium of 7 common phenotypes.[25] Full details including quality control (QC) measures are given in our primary GWAS paper.[9] We excluded markers where (1) Hardy–Weinberg equilibrium (HWE) controls $P < 0.001$, (2) HWE cases $P < 0.00001$, (3) MAF in either cases or controls < 0.01, (4) the genotyping call rate in either cases or controls was < 0.985. The quality of the genotyping for the markers we report to be associated with schizophrenia was verified by inspection of the genotype clusters. Follow-up genotyping in the additional 163 UK cases, Irish, Bulgarian, German (Munich collection) and Swedish samples was performed using Sequenom iPLEX Gold chemistry on the Sequenom MassARRAY genotyping platform in Cardiff. Cross-platform studies were performed for 61 SNPs from the chromosome 10 linkage region by re-genotyping on average 179 samples from the 1958 birth cohort sample set (used in the WTCCC study[25]) on the Sequenom platform. Sequenom assays were also validated by genotyping all follow-up SNPs in the 90 Centre d'Etude du Polymorphisme Humain (CEPH) individuals from the International HapMap project. The Chinese, Japanese, Bonn, Israel and US/Australia samples were genotyped by those groups as previously described and blind to the results from our GWAS.[9]

Statistical analysis

In the GWAS sample, the primary test of association was the Armitage trend test (1 df). For meta-analyses of the replication samples and combined samples, we used the Cochran–Mantel–Haenszel test as implemented in PLINK.[27] HWE was calculated by a goodness-of-fit test.

Results

QC analysis

QC genotyping for 61 SNPs on the Sequenom platform identified 6 discrepant genotypes from a total of 10890 giving a discrepancy rate of 0.06% between Sequenom and Affymetrix platforms. The average call rate in the GWAS sample for the SNPs in our target region was 98.9%. The particular SNP emergent as showing strongest evidence for association in this study (rs17101921) was re-genotyped on the Sequenom platform in all GWAS schizophrenia cases and 1426 GWAS controls. Two discrepancies were identified in the case sample. Both were from the only two minor allele homozygous genotypes called in either cases or in controls on the Affymetrix platform. These were assigned heterozygous genotypes on the Sequenom platform. Genotyping of rs17101921 for the two discrepant samples was repeated with a SNaPshot genotyping assay, proving the Sequenom platform to be correct (hence these individuals are called as heterozygotes in all analyses reported for this SNP). Complete concordance was observed between Affymetrix and Sequenom platforms for 1426 GWAS controls. No other minor allele homozygotes were observed for rs17101921 on the Affymetrix platform for any other schizophrenia GWAS cases or any of the 2937 GWAS controls. One WTCCC bipolar case was scored as a minor allele homozygote on the Affymetrix platform and for the purpose of this analysis, this individual was excluded. For the Bulgarian, German,

and Irish replication samples typed on the Sequenom platform, average call rates for all chromosome 10 SNPs typed were 98.7, 98.3 and 98.5%, respectively. Call rates for rs17101921 in the Bulgarian, Munich, Dublin, Bonn, US/Australian, China, Japan and Swedish samples were 97.8, 95.3, 99.1, 100, 99.9, 98.1, 98.0 and 98.3%, respectively. Concordance for all SNPs typed through the same 90 CEPH individuals used in the International HapMap was 99.9%.

Association in the UK sample

Following QC, we used a total of 3606 SNPs (Figure 2) of which 40 showed evidence for association in the GWAS sample $P<0.01$. (Supplementary Table 1). Of these 40 markers, 22 remained significant at $P<0.01$ in the full UK sample (Supplementary Table 1).

Replication studies

Markers that showed nominally significant evidence for association ($P<0.01$) in the extended UK sample ($n=22$) were genotyped in samples from Ireland, Bulgaria and Germany. Only one SNP showed nominally significant evidence for association to the same allele at $P<0.05$ in the meta-analysis of the replication samples (Supplementary Table 2). This was rs17101921 ($P=0.002$, one-tailed) located at chr10: 123 143 285 (UCSC March 2006) ~85 kb 3′ of *FGFR2* (Table 1).

In the second set of replication samples from Sweden, United States/Australia, China, Japan, Germany (Bonn) and Israel (Tables 1 and 2), rs17101921 again showed significant association to the same allele ($P=0.011$, one-tailed) and when all replication samples were combined (total 6806 cases, 10 102 controls), this marker was associated with schizophrenia ($P=0.0009$, one-tailed) with an OR of 1.17 (95% CI 1.06–1.29). When this was subjected to correction for the SNPs taken forward for replication, this finding remained significant, $P=0.015$ (based on $\alpha=0.05$ there were 17.1 effective tests from the 22 SNPs calculated as per Moskvina and Schmidt[28]). The combined GWAS and replication samples yielded $P=0.0002$, OR 1.19 (95% CI 1.09–1.31) (Table 1).

Our GWAS sample contained a total of 108 unrelated individuals with an affected sibling who had been included in our previous linkage study.[15] In addition we had access to 91 unrelated Swedish cases with an affected sibling who had also been included in our linkage study,[15] plus a further 49 unrelated Swedish cases with an affected sibling who had been recruited for but did not participate in the linkage study. One of the replication samples (United States/Australia) contained a subset of individuals with an affected sibling ($n=289$) that were also included in a linkage study[21] that had shown linkage to chromosome 10q25–q26. We hypothesized that an increased effect size at rs17101921 would be observed in these familial cases. The effect size at rs17101921 was increased in the familial cases from the UK (OR 1.92 (95% CI 1.19–2.41)) and Sweden (OR 2.85 (95% CI 1.39–5.85)) which formed the majority of the sample in which we obtained evidence for linkage. In the US/Australian replication sample, the effect was actually in the opposite direction, but in the subset of familial cases, the trend for association was in the

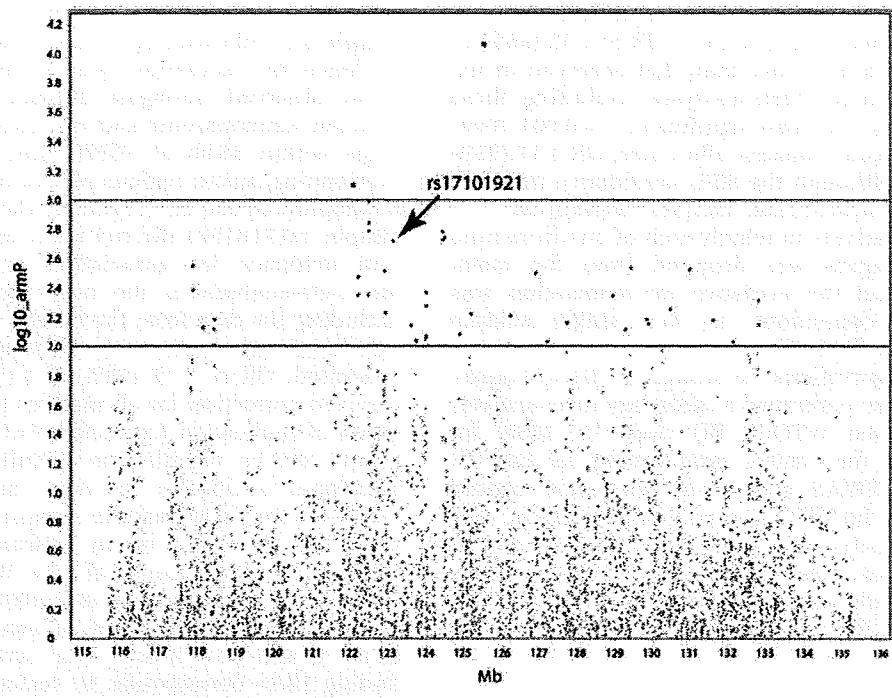

Figure 2 UK GWAS *P*-values across chromosome 10 linkage region. position_MB; position in megabases, log10_armp; armitage trend test −log10 *P*-values. Top horizontal line corresponds to $P<0.001$, bottom horizontal line corresponds to $P<0.01$.

Table 1 Meta-analyses of rs17101921

rs17101921	*No. of cases*	*No. of controls*	*CMH_P*	*CMH_OR*	*95% CI*	*95% CI*
Rep 1	1664	3541	0.0020	1.48	1.13	1.94
Rep all	6806	10102	0.0009	1.17	1.06	1.29
Meta all + UK	7448	13039	0.0002	1.19	1.09	1.31

Rep 1: first set of replication samples from Ireland, Germany (Munich) and Bulgaria. Rep all: meta-analysis of above, plus additional samples from United States/Australia, Germany (Bonn), China, Japan, Israel and Sweden. Meta all + UK = meta-analysis of all samples including UK.

Table 2 Association analysis of rs17101921

rs17101921	*No. of cases*	*No. of controls*	*CON freq 1*	*CASE freq 1*	*Allelic P*	*OR*	*95% CI*	*95% CI*
UK GWAS	479	2937	0.020	0.035	0.006	1.73	1.17	2.55
UK extended	642	2937	0.020	0.033	0.003	0.69	1.19	2.41
Ireland	295	983	0.019	0.045	0.0004	2.34	1.43	3.83
Bulgaria	611	661	0.016	0.028	0.019	1.80	1.04	3.12
German Munich	758	1897	0.024	0.025	0.401	1.05	0.71	1.56
Sweden	140	205	0.027	0.074	0.002	2.85	1.39	5.85
German Bonn	735	1036	0.027	0.020	0.240	0.76	0.49	1.19
United States/Australia	1744	1938	0.028	0.028	0.924	0.99	0.75	1.30
China	1034	1034	0.138	0.165	0.008	1.24	1.04	1.47
Japan	748	831	0.209	0.224	0.157	1.09	0.92	1.29
Israel	741	1517	0.017	0.020	0.196	1.22	0.78	1.92

Abbreviations: CI, confidence interval; GWAS, genome-wide association study.
CON freq 1 = minor allele frequency in controls, CASE freq 1 = minor allele frequency in cases. Allelic *P*-values are one-tailed where OR is in the same direction as the GWAS sample. OR: odds ratio with 95% CI.

same direction as the GWAS sample, OR 1.12 (95% CI 0.68–1.85). Meta-analyses across all three chromosome 10 linked familial samples showed significant association, $P = 0.045$ (two-tailed) with an OR of 1.52 (95% CI 1.02–2.27) which is greater than that observed in the sample as a whole. Meta-analyses excluding these familial samples was also significant, $P = 0.001$ (two-tailed) and showed a smaller effect size, OR 1.17 (95% CI 1.06–1.29), although the 95% confidence intervals for familial and nonfamilial samples overlapped.

Sensitivity analysis in which each of one from nine replication samples was dropped from the meta-analysis revealed the evidence for association was not critically dependent on any single sample (Supplementary Table 3).

Given the observations of linkage of BD to 10q25–q26,[17,18,20] we also performed a secondary meta-analysis that included the WTCCC BD cases ($n = 1865$) for rs17101921. As the control sample used for the UK schizophrenia GWAS study was the same control sample used in the WTCCC bipolar GWAS study, they were included only once. Inclusion of the BD cases in the meta-analysis (total 9313 cases, 13 039 controls) increased the evidence for association with rs17101921, $P = 7.80 \times 10^{-5}$, OR 1.20 (95% CI 1.10–1.31).

Discussion

There are seven reports of linkage between chromosome 10q25–q26 and either schizophrenia or BD, but no susceptibility genes within this region have yet been identified. Aiming to map susceptibility variants, we used GWAS data from a UK case–control sample and followed up those variants that provided evidence for association in up to two sample series.

We observed strongest evidence for association between schizophrenia and rs17101921, a marker that maps within 85 kb of *FGFR2* and is located in the overlapping linked regions of five of seven studies of schizophrenia and BD (Figure 1). Clearly, in our GWAS sample, rs17101921 did not show region-wide significant evidence for association and thus although the meta-analysis of the nine replication samples, excluding the data from the GWAS sample, showed a significant association with schizophrenia ($P = 0.0009$, one-tailed, OR of 1.17 (95% CI 1.06–1.29)), and this survived correction for all markers taken into the first phase of replication (corrected $P = 0.015$), the finding cannot yet be regarded as definitive, and requires additional evaluation in even larger studies. The results of the meta-analysis suggest a similar conclusion. On the basis of an estimated genome-wide threshold for significance of 7.2×10^{-8} [29] and that our target region comprises about 2/300ths of the genome, the region-wide equivalent of genome-wide significance would be 1×10^{-5}. Our overall meta-analysis finding then corresponds to region-wide suggestive rather than significant evidence.

The question of whether and to what extent this locus might account for the evidence for linkage to

10q25–q26 is difficult to determine given that the true effect size at this locus remains unknown pending discovery of the underlying functional variant and that the true effect size of the linkage is difficult to measure in an unbiased way. However, we note that the effect size at rs17101921 was greater in the familial cases, which formed the majority of the sample in which we obtained evidence for linkage, than that observed in the replication samples, which provides the most unbiased estimate of the true effect size in the remaining samples. Of course, at this stage, we cannot exclude the possibility that the linkage observed at 10q25–26 reflects the combined effects of *FGFR2* and another locus or loci within this region. We also note that the evidence for association was increased with the addition of 1865 BD cases from the United Kingdom. Given that the increase in evidence was modest, and that BD was itself significantly associated in the WTCCC sample ($P = 3.5 \times 10^{-2}$), and the controls are shared with the schizophrenia sample, the hypothesis that this SNP is another example of an association across diagnostic boundaries should be taken with additional caution.

In terms of the possible functional implications of the association we report, the nearest gene to rs17101921 is *FGFR2* (~85 kb 3′, UCSC March 2006 freeze). We note here from two recent studies of gene expression that the majority of significant *cis*-acting SNP variants occur within 100 kb (either 3′ or 5′) of the interrogated expression probe and also that significant *cis*-acting variants are observed as far as 800 kb away.[30,31] On the basis of *in silico* data, rs17101921 does not appear to reside in a highly conserved or putative functional region. Of additional interest is the variable frequency of the risk allele. In our replication samples, the frequency of the risk allele in Chinese controls is 14% and in Japanese controls, 21% compared to only ~2% in our Caucasian samples. HapMap populations show similar frequencies, with the CEPH sample being nonpolymorphic and the Chinese and Japanese samples showing a much higher frequency. The Yoruban population is also nonpolymorphic, suggesting perhaps that this variant could be under selection.

FGFR2 is a reasonable functional candidate gene for schizophrenia. Of its multiple functions, perhaps of most plausible relevance to schizophrenia is that it plays roles in presynaptic organization early in development, neuroprotection and repair in adulthood and oligodendrocyte development.[32,33] Recent evidence provides some support for the involvement of the fibroblast growth factor (FGF) system in psychiatric disorders (reviewed in[34,35]), and, more specifically, some support for the involvement of FGF signaling in schizophrenia. Thus, Hashimoto *et al.*[36] reported altered levels of FGF-2 in blood from drug-free schizophrenia patients, whereas Gaughran *et al.*[37] reported increased measures of *FGFR1* mRNA in schizophrenic brain. Interestingly, mice homozygous for a dominant-negative mutation at *FGFR1* showed increased levels of dopamine in the striatum and impaired prepulse inhibition.[38] Although these studies provide some plausibility that altered *FGFR2* function might contribute to schizophrenia, our study provides no direct evidence for this. Further studies of genetic association are required, and if confirmed, investigation into the biological significance of the association.

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Identification of loci associated with schizophrenia by genome-wide association and follow-up

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We carried out a genome-wide association study of schizophrenia (479 cases, 2,937 controls) and tested loci with $P < 10^{-5}$ in up to 16,726 additional subjects. Of 12 loci followed up, 3 had strong independent support ($P < 5 \times 10^{-4}$), and the overall pattern of replication was unlikely to occur by chance ($P = 9 \times 10^{-8}$). Meta-analysis provided strongest evidence for association around *ZNF804A* ($P = 1.61 \times 10^{-7}$) and this strengthened when the affected phenotype included bipolar disorder ($P = 9.96 \times 10^{-9}$).

Schizophrenia has a lifetime risk of approximately 1% and is characterized by delusions, hallucinations, altered cognition, emotional reactivity and disorganized behavior. Genetic factors account for more than 80% of the variance in susceptibility[1], and risk likely results from multiple loci of small effect[2]. Positional cloning studies have identified a small number of promising susceptibility genes[3]. None are undisputed, but the apparent effect sizes are small (OR < 1.5) and individual studies are generally underpowered to replicate them.

Genome-wide association (GWA) methods have proven successful for other disorders in identifying susceptibility genes with effect sizes expected for schizophrenia[4–7]. Seeking new susceptibility genes for schizophrenia, we used a multistage association study design founded upon a GWA study. Our GWA cases ($n = 479$) were drawn from a total sample of 642 individuals meeting DSM-IV (Diagnostic and Statistical Manual of Mental Disorders IV) criteria for schizophrenia. In concert with the Wellcome Trust Case Control Consortium study (WTCCC)[4], we genotyped the 479 cases with the Affymetrix GeneChip 500K Mapping Array and compared the allele frequencies with 2,937 controls from that study. The chip contains 500,568 SNPs, of which 362,532 passed the quality control measures we adopted (**Supplementary Methods** online). Association analyses were undertaken using 1-degree-of-freedom trend tests. From these, we calculated a genomic control factor (λ)[8] of 1.08. This is unlikely to reflect stratification, as it was not appreciably reduced (range 1.08–1.07) by analyses regressing on up to the first four components derived from multi-dimensional scaling (**Supplementary Figs. 1** and **2** online). Thus, our primary analysis is based on unadjusted P values, although we also present conservative λ-adjusted P values in **Table 1** for reference (**Supplementary Note** online).

We followed the WTCCC in considering thresholds of $P < 1 \times 10^{-5}$ and $P < 5 \times 10^{-7}$ as moderately strong and strong evidence for association, respectively[4]. Loci ($n = 12$) with $P < 1 \times 10^{-5}$ (**Table 1**) were selected for follow-up. For 11 of these, we tested the SNP that was most strongly associated in the UK sample (**Supplementary Table 1** online). For the remaining locus (chr. 11, ~132.1 Mb), we used the second strongest SNP, as the strongest did not give robust genotypes. The primary analysis for replication samples (and meta-analysis) was the Cochran-Mantel-Haenszel (CMH) test, but we also present (**Table 1**) a P value based meta-analysis to allow adjustment for λ (**Supplementary Note**). In replication sample 1 (1,664 cases, 3,541 controls; described in **Supplementary Table 2** online and **Supplementary Methods**), we replicated the association observed in the GWA sample (**Table 1**) for 6 of the 12 SNPs. We then genotyped these 6 SNPs in replication sample 2 (4,143 cases, 6,515 controls).

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Table 1 Loci selected for follow-up analysis

			Combined UK samples					Replication 1		Replication 1+2		Meta SZ			
			Cases n = 642 Controls n = 2,937					Cases n = 1,664 Controls n = 3,541		Cases n = 6,666 Controls n = 9,897		Cases n = 7,308 Controls n = 12,834			
Chr./Mb	SNP	Risk allele	SZ	CON	ATT(P)	Adj(P)	OR	CMH(P)	OR	CMH(P)	OR	CMH(P)	Meta-Adj	OR	Locus
2/185.5	rs1344706	T	0.66	0.59	7.08×10^{-7}	1.83×10^{-6}	1.38	0.026	1.09	9.25×10^{-5}	1.09	1.61×10^{-7}	1.95×10^{-7}	1.12	*ZNF804A*
11/29.1	rs1602565	C	0.15	0.11	7.81×10^{-6}	1.70×10^{-5}	1.49	0.005	1.19	3.22×10^{-4}	1.12	2.99×10^{-6}	3.02×10^{-6}	1.16	Intergenic
16/13.0	rs7192086	T	0.30	0.24	3.32×10^{-5}	6.52×10^{-5}	1.33	0.018	1.11	5.10×10^{-4}	1.09	6.08×10^{-6}	1.34×10^{-5}	1.12	Intergenic
11/132.1	rs3016384	C	0.56	0.49	5.82×10^{-5}	1.10×10^{-4}	1.29	0.012	1.10	0.016	1.05	5.63×10^{-4}	1.11×10^{-4}	1.08	*OPCML*
16/52.2	rs9922369	A	0.05	0.03	8.05×10^{-7}	2.05×10^{-6}	2.06	0.015	1.31	0.029	1.14	4.54×10^{-4}	5.01×10^{-6}	1.24	*RPGRIP1L*
12/116.2	rs6490121	G	0.40	0.34	4.33×10^{-6}	9.82×10^{-6}	1.33	0.044	1.08	0.992	1.00	0.109	5.51×10^{-3}	1.04	*NOS1*
2/144.3	rs2890738	A	0.41	0.33	4.96×10^{-9}	1.83×10^{-8}	1.44	0.249	1.03	–	–	–	–	–	Intergenic
3/134.5	rs7624858	A	0.44	0.37	1.15×10^{-4}	2.07×10^{-4}	1.27	0.113	1.06	–	–	–	–	–	*TMEM108*
5/138.5	rs17131938	A	0.07	0.04	2.94×10^{-4}	4.94×10^{-4}	1.64	0.091	0.81	–	–	–	–	–	*SIL1*
10/5.6	rs4750519	T	0.48	0.41	1.07×10^{-4}	1.93×10^{-4}	1.27	0.612	0.98	–	–	–	–	–	Intergenic
15/94.0	rs8031294	T	0.51	0.42	2.29×10^{-5}	4.62×10^{-5}	1.30	0.311	1.02	–	–	–	–	–	Intergenic
18/9.0	rs1893146	A	0.16	0.11	5.40×10^{-7}	1.42×10^{-6}	1.55	0.102	0.89	–	–	–	–	–	Intergenic

SZ and CON; allele frequency in schizophrenia and controls. ATT(P), trend test P value; Adj(P), genomic control adjusted P value; CMH(P), Cochran-Mantel-Haenszel P value; Meta-Adj, genomic control adjusted meta-analysis P value.

The full replication dataset (replication samples 1 + 2; **Table 1**) provided strong independent support for schizophrenia susceptibility variants at 2q32.1 in *ZNF804A* ($P = 9.25 \times 10^{-5}$) and at intergenic regions on 11p14.1 at 29.1 Mb ($P = 3.22 \times 10^{-4}$) and 16p13.12 at ~13 Mb ($P = 5.10 \times 10^{-4}$). Two additional loci, one within the *RPGRIP1L* locus on 16q12.2 at 52.2 Mb and one within *OPCML* at 11q25 (132.1 Mb), remained nominally significant.

The distribution of replication P values is highly unlikely ($P = 9 \times 10^{-8}$) to have occurred by chance, indicating that the GWA threshold $P < 10^{-5}$ enriched for true associations. However, that analysis does not allow any single locus to be assessed in the context of a potential genome-wide study of all samples, nor does it allow for future follow up of lower-order signals. Thus, we combined the data across all samples (**Table 1**), and found that the *ZNF804A* locus ($P = 1.61 \times 10^{-7}$) surpassed the $P \lesssim 5 \times 10^{-7}$ benchmark corresponding to strong evidence for association, whereas the regions on chromosomes 11 (29.1 Mb) and 16 (13.0 Mb) showed moderately strong evidence (**Table 1**).

There is evidence that schizophrenia and bipolar disorder have some risk factors in common[9]. Thus, as a secondary analysis, we added genotypes from the bipolar cases (n = 1,865) from the WTCCC study to the UK schizophrenia cases to create a large UK psychosis sample for inclusion in the meta-analysis. (See **Table 2**; note that the controls for the schizophrenia and bipolar cases are shared, so the individual associations are not independent. In the meta-analysis, the controls were used only once.) We did not find any evidence for shared risk for most of the loci, but for *ZNF804A*, the evidence was substantially stronger ($P = 9.96 \times 10^{-9}$), suggesting that alleles in the vicinity of *ZNF804A* influence risk to a broader psychosis phenotype. When we combined the data from the WTCCC phenotypes other than bipolar disorder in the meta-analysis with the UK schizophrenia sample, the evidence for association did not strengthen, suggesting that the effect observed from addition of bipolar samples was not simply a consequence of comparing a larger sample with the WTCCC controls.

Our samples included individuals from China and Japan as well as Ashkenazi Jews and outbred Europeans; therefore, the meta-analysis may have been influenced by heterogeneity. In the full replication sample, five of the six loci tested yielded no evidence for heterogeneity (Breslow-Day $P_{min} = 0.10$), and removal of any individual replication sample did not markedly alter the effect size (**Supplementary Table 3** online). The exception was rs9922369, which was monomorphic in Japan and China and had a significantly higher ($P = 8 \times 10^{-10}$

Table 2 Combined schizophrenia and bipolar analysis

						UK SZ		UK BP		Meta SZ + BP	
			Allele freq.			Cases n = 642, Controls n = 2,937		Cases n = 1,865, Controls n = 2,937		Cases n = 9,173, Controls n = 12,834	
Chr./Mb	SNP	Risk allele	SZ	BP	CON	ATT(P)	OR	ATT(P)	OR	CMH(P)	OR
2/185.5	rs1344706	T	0.66	0.62	0.59	7.08×10^{-7}	1.38	4.07×10^{-4}	1.16	9.96×10^{-9}	1.12
11/29.1	rs1602565	C	0.15	0.12	0.11	7.81×10^{-6}	1.49	0.055	1.14	4.26×10^{-6}	1.15
12/116.2	rs6490121	G	0.40	0.35	0.34	4.33×10^{-6}	1.33	0.168	1.06	0.124	1.03
16/52.2	rs9922369	A	0.05	0.03	0.03	8.05×10^{-7}	2.06	0.261	1.15	0.002	1.20
16/13.0	rs7192086	T	0.30	0.25	0.24	3.32×10^{-5}	1.33	0.206	1.06	2.56×10^{-5}	1.10
11/132.1	rs3016384	C	0.56	0.51	0.49	5.82×10^{-5}	1.29	0.057	1.08	4.43×10^{-4}	1.07

SZ, schizophrenia; BP, bipolar; CON, control; ATT(P), trend test P value; meta SZ+BP, meta-analysis for all schizophrenia and bipolar samples reported in this study; CMH(P), Cochran-Mantel-Haenszel P value.