No association was found between schizophrenia and controls. Our sample size has a power of 0.80 to detect significant associations between each 'tag SNP' and schizophrenia, assuming a genotype relative risk (GRR) of 1.42–1.57 under a multiplicative model of inheritance. In contrast, all 'tag SNPs' (SNP3–5) were associated with METH use disorder in the allelic, haplotypic and/or genotypic analyses (Tables 1 and 2). Our METH use disorder samples are expected to yield power of 0.80 to detect significant association, assuming GRR of 1.58–1.75.

For further interpretation of these associations, we also included an explorative analysis of clinical subgroups and gender effects for the following reasons: our subjects with METH-induced psychosis were (1) the majority of our METH samples, this condition would be over-represented in our samples of METH use disorder, and (2) unmatched gender samples for METH use disorder (male = 145 and female = 32). Consequently, no common results among clinical subgroups or gender were obtained. However, SNP3 was associated with female METH use samples, and SNP5 was associated with total METH use disorder and METH-induced psychosis in male samples (Table 3). Global haplotypic analyses showed only trends for significance in male METH use disorder samples (global *P*-value = 0.0536) and male METH-induced psychosis samples (global *P*-value = 0.105) (Supplemental Table S2).

Discussion

A possible association of 'tag SNPs' in the *ARRB2* gene was obtained in the patients with METH use disorder but not with schizophrenia patients.

The results from the LD evaluation using the TAGGER program indicate that three 'tag SNPs' could represent the entire 5′-flanking regions and most 3′ regions. We speculate that an actual susceptibility variant of METH use disorder may exist within the LD region in these significant SNPs, because these 'tag SNPs' may be only markers with no functional effects (SNP3: synonymous substitution, SNP4 and SNP5: located in 3′ downstream of ARRB2). Further investigations including mutation scan and functional analysis will be required.

Our METH use disorder sample had several limitations, described in the *Results* section. In terms of clinical subgroups, because there was no high power association between ARRB2 and schizophrenia, the association between ARRB2 and total METH use disorder is perhaps not due to

Table 1: Association analyses of 'tag single nucleotide polymorphisms (SNPs)' in β-arrestin 2 (ARRB2) with schizophrenia and methamphetamine (METH) use disorder

			Genotype*			*P*-values†	
tag SNPs	Phenotype	Number	M/M	M/m	m/m	Genotype	Allele
SNP3 rs1045280 (T>C)	Schizophrenia	547	418	119	10	0.753	0.859
	METH use disorder	177	117	54	6	**0.0118**	**0.00351**
	Controls	546	417	122	7		
SNP4 rs2036657 (A>G)	Schizophrenia	547	430	109	8	0.871	0.596
	METH use disorder	177	129	44	4	0.117	**0.0431**
	Controls	546	436	103	7		
SNP5 rs4790694 (C>A)	Schizophrenia	547	460	85	2	0.703	0.382
	METH use disorder	177	138	39	0	**0.0167**	**0.0202**
	Controls	546	470	74	2		

*M, major allele; m, minor allele.

†Bold numbers represent significant *P*-values.

Table 2: Haplotypic analysis of three 'tag single nucleotide polymorphisms (SNPs)' (SNP3–5)

			Frequency (%)		
Phenotype	Global *P*-values	Marker haplotype	Case	Control	Individual *P*-values*
Schizophrenia	0.405	TAC	84.8	85.3	0.716
		CGA	6.03	5.19	0.400
METH use disorder	**0.0175**	TAC	80.2	85.4	**0.0194**
		CGA	8.60	5.20	**0.0210**

*Bold numbers represent significant *P*-values.

Table 3: Explorative analysis of clinical subgroups and gender in methamphetamine (METH) use disorder

tag SNPs	Phenotype	Number	Genotype*			*P*-values†	
			M/M	M/m	m/m	Genotype	Allele
SNP3	Male METH use disorder	145	99	40	6	0.224	0.152
	psychosis	138	96	38	4	0.523	0.340
	dependence	7	3	2	2	NA	NA
	Male control	255	187	64	4		
	Female METH use disorder	32	18	14	0	**0.0123**	**0.0112**
	psychosis	26	17	9	0	0.211	0.172
	dependence	6	1	5	0	NA	NA
	Female control	291	230	58	3		
SNP4	Male METH use disorder	145	108	33	4	0.466	0.375
	psychosis	138	104	31	3	0.695	0.558
	dependence	7	4	2	1	NA	NA
	Male control	255	197	55	3		
	Female METH use disorder	32	21	11	0	0.0652	0.0595
	psychosis	26	19	7	0	0.423	0.375
	dependence	6	2	4	0	NA	NA
	Female control	291	239	48	4		
SNP5	Male METH use disorder	145	113	32	0	**0.0174**	**0.0231**
	psychosis	138	108	30	0	**0.0224**	**0.0302**
	dependence	7	5	2	0	NA	NA
	Male control	255	223	31	1		
	Female METH use disorder	32	25	7	0	0.374	0.371
	psychosis	26	22	4	0	1	0.992
	dependence	6	3	3	0	NA	NA
	Female control	291	247	43	1		

*M, major allele; m, minor allele; NA, not analyzed.
†Bold numbers represent significant *P*-values.

spurious comorbid METH-induced psychosis, which may share the pathophysiology of susceptibility with schizophrenia (sensitization phenomena) (Ujike & Sato 2004). Thus, all significant SNPs might be closely associated with METH use disorder.

However, a recent investigation suggests that METH-induced psychosis has heavier family loading, probably heavier genetic loading than METH users without psychosis (Chen *et al.* 2005). The authors of that study suggested it is likely that dopaminergic abnormalities differ between METH used disorder with and without psychosis.

Furthermore, gender effects of METH use disorder and AKT/GSK signaling were also reported (Jang *et al.* 1997; Znamensky *et al.* 2003). We recognize that a larger sample will be required for the confirmation of clinical subgroups and a gender effect, because owing to the small sample size of METH subjects without psychosis and female METH subjects our explorative analysis mainly reflected the results from male subjects with psychosis. Therefore, our speculation is based mainly on the results of these subjects. Even taking such a conservative view of this explorative analysis, we consider that ARRB2 may be associated with METH-induced psychosis in males and that among the significant SNPs, SNP5 is the most plausible candidate variant. Our findings from comparing genotype frequencies of SNPs between METH-induced psychosis in males and in male controls would seem to indirectly support the possibility of a different genetic background for METH-induced psychosis (Chen *et al.* 2005). Chen *et al.* (2005) also reported significant differences in morbid risk, when the METH-induced psychosis subjects were divided into clinical subgroups of prolonged METH psychosis and brief METH psychosis. We could not perform association analyses between METH use disorders with and without psychosis in this study due to the small sample size and the differences in the inclusion criteria for prognosis of METH-induced psychosis (Morita *et al.* 2005). Meanwhile, the results from the haplotypic analyses, which showed no association with METH use disorder or METH-induced psychosis in male samples, may have been due to

type II error, because a power simulation showed that haplotypic analysis can decrease the statistical power of an association study [if an actual causal variant (or variant in absolute LD with that variant) is directly observed] (Bader 2001).

Recent *in vivo* studies suggest that the AKT/GSK3 signaling cascade is an attractive candidate factor for schizophrenia and METH use disorder (Beaulieu *et al.* 2004, 2005; Emamian *et al.* 2004). We have performed genetic association studies of AKT1 and GSK3B with schizophrenia (Ikeda *et al.* 2004, 2005b) and METH use disorder (Ikeda *et al.* 2005c) (for GSK3B, no association was obtained from subjects with METH use disorder; M. Ikeda, N. Ozaki, N. Iwata & JGIDA, unpublished observations). Summarizing these results, AKT1 (but not GSK3B and ARRB2) showed a significant association with schizophrenia, and AKT1 and ARRB2 (but not GSK3B) were associated for METH use disorder. To evaluate whether each risk SNP would be an independent risk factor or have interactions with each SNP in these genes, we analyzed the gene–gene interactions with the use of the Multifactor Dimensionality Reduction (MDR) method (Hahn *et al.* 2003). In this analysis, we considered two-locus interactions through four-locus interactions and analyzed six SNPs in AKT1, two SNPs in GSK3B and three SNPs in ARRB2. Three hundred and seventy-one schizophrenics, 161 METH use disorder subjects and 339 controls were tested, all of whom were identical in each study. However, no interactions were obtained with schizophrenia or METH use disorder (and METH-induced psychosis in males) (data not shown). Further studies will be required for conclusive results; however, these results indicate that the risk SNPs or haplotypes in AKT1 for schizophrenia, and those in AKT1 and ARRB2 for METH use disorder may attribute independently to these disorders.

In conclusion, our findings suggest that ARRB2 may play a role in the development of METH use disorder but not schizophrenia in the Japanese population. However, this positive finding is a preliminary one, given the several limitations of our sample and the fact that we did not perform genomic control to exclude population stratification. We must also consider the possibility that our findings merely demonstrated the pharmacogenetic fact that individuals metabolize drugs differently, and the results were not relevant to the genetics of psychoses but rather to their treatment. It will be necessary to validate or replicate our associations in other population samples.

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Supplementary material

Table S1. Pairwise linkage disequilibrium matrices and 'tag SNPs' in ARRB2.
Table S2. Explorative haplotypic analysis of clinical subgroups and gender in METH use disorder.
These materials are available as part of the online article from http://www.blackwell-synergy.com

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LETTERS TO THE EDITOR

Support for association of the *PPP3CC* gene with schizophrenia

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Calcineurin is a calcium-dependent protein phosphatase that plays an important role in cellular responses and calcium signal transduction.[1] Several studies have suggested that calcineurin is one of the key molecules in signal transduction in the brain and that dysfunction of calcineurin signaling could be linked to schizophrenia.[2,3] Calcineurin is a heteromeric protein complex consisting of a catalytic subunit (calcineurin A) and a regulatory subunit (calcineurin B).[1,4] *PPP3CC* encodes the calcineurin γ-catalytic subunit and is located on chromosome 8p21.3 within a few cM of markers reported to be linked to schizophrenia.[5–7,10] Gerber *et al.*[8] reported genetic associations of the *PPP3CC* gene with schizophrenia in populations from the United States and South Africa. However, only one replication study has been published, and these associations were not confirmed in 457 Japanese schizophrenic patients and 429 control subjects.[9] HapMap data indicated that a haplotype block spans almost the entire *PPP3CC* region in Japanese and European populations. The single nucleotide polymorphism (SNP) haplotype reported to be associated with schizophrenia by Gerber *et al.*[8] is located in the haplotype block. Therefore, we examined the associations in a large case–control study of 1645 schizophrenic patients and 1673 control subjects. This sample size has a power >0.98 to replicate the haplotypic association with the same magnitude as that reported by Gerber *et al.*,[8] assuming an α value $=0.05$, two-tailed, a haplotype relative risk of 1.23, and haplotype frequency of 0.26 or effect size of 0.1. The haplotype frequency in the Japanese population was reported by Kinoshita *et al.*[9]

All subjects were of Japanese descent and were recruited from the main island of Japan. The study included 1645 unrelated patients with schizophrenia (mean age $\pm$ s.d., 48.2 ± 14.5 years; 899 men and 745 women) diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). Control subjects were 1673 mentally healthy unrelated subjects (age, 47.9 ± 14.3, 886 men and 787 women) without self-reported family histories of mental illness within second-degree relatives. The subjects studied by Kinoshita *et al.*[9] were not included in the present study. 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 and all participants provided written informed consent. To rule out population stratification between patients and controls in the present study, 35 SNPs that are not in linkage disequilibrium (LD) with each other were genotyped in all samples and analyzed with the STRUCTURE program 2.0.[5] No stratification was observed.

We genotyped five SNPs. SNP1 (rs10108011, CC21 in Gerber *et al.*[8]) and SNP2 (rs2461491, CCS3 in Gerber *et al.*[8]) were selected because Gerber *et al.*[8] reported nominally significant allelic association of these SNPs with schizophrenia. SNP4 (rs2449340), SNP3 (rs2461490) and SNP5 (rs1116085) were genotyped to distinguish common haplotypes with frequencies $\geqslant 5\%$ in the haplotype block based on HapMap data of the Japanese population. SNPs were genotyped with the TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA) and ABI PRISM 7900HT Sequence Detection System (Applied Biosystems).

Deviation from predicted Hardy–Weinberg frequency was examined by χ^2-test. Individual allelic and genotypic associations were examined by Fisher's exact test. LD between polymorphisms and haplotypic associations were evaluated with Haploview software version 3.32.[6] To deal with multiple testing, allelic associations were evaluated by permutation tests implemented in Haploview. The genotype distributions were evaluated by the Cochran–Armitage test without correction for multiple testing.

The genotype and allele distributions of the five SNPs in the patient group and control group are shown in Table 1. Distributions of these SNPs did not differ significantly from Hardy–Weinberg equilibrium. All five SNPs showed nominally significant allelic associations with schizophrenia and permutation tests revealed significant allelic associations of SNP1 ($P=0.012$), SNP3 ($P=0.005$) and SNP4 ($P=0.013$) with schizophrenia. The genotype distributions suggest that the minor allele of each SNP is likely to have an additive effect in the susceptibility to schizophrenia. These five SNPs are in LD; however, the LD is not complete. Therefore, these associations were not caused by a single SNP in the present study. As shown in Table 2, there were only two common haplotypes constructed from these SNPs. The most common haplotype in the control group was observed less frequently in the patient group ($P=0.034$) and the second most common

Table 1 Case-control comparisons of SNPs

Polymorphism (NCBI ID)	*Subjects*	n	*Genotype count (frequency)*			P[a]	*Allele count (frequency)*		P[b] (P[c])	*Odds ratio (95% CI)*	*HWE* P
			AA	AG	GG		A	G			
SNP1(CC21)	Affected	1639	828 (0.51)	644 (0.39)	167 (0.10)		2300 (0.70)	978 (0.30)	0.012	1.15	0.013
(rs10108011)	Controls	1665	897 (0.54)	636 (0.38)	132 (0.08)	0.013	2430 (0.73)	900 (0.27)	(0.030)	(1.03–1.28)	0.197
			GG	GA	AA		G	A			
SNP2(CCS3)	Affected	1645	568 (0.35)	781 (0.47)	296 (0.18)		1917 (0.58)	1373 (0.42)	0.076	1.09	0.335
(rs2461491)	Controls	1673	617 (0.37)	788 (0.47)	268 (0.16)	0.076	2022 (0.60)	1324 (0.40)	(0.149)	(0.99–1.21)	0.536
			CC	CG	GG		C	G			
SNP3	Affected	1636	690 (0.42)	731 (0.45)	215 (0.13)		2111 (0.65)	1161 (0.35)	0.005	1.16	0.330
(rs2461490)	Controls	1655	773 (0.47)	698 (0.42)	184 (0.11)	0.006	2244 (0.68)	1066 (0.32)	(0.015)	(1.05–1.28)	0.165
			GG	GT	TT		G	T			
SNP4	Affected	1639	719 (0.44)	716 (0.44)	204 (0.12)		2154 (0.66)	1124 (0.34)	0.013	1.14	0.216
(rs2449340)	Controls	1665	792 (0.48)	700 (0.42)	173 (0.10)	0.014	2284 (0.69)	1046 (0.31)	(0.038)	(1.03–1.26)	0.321
			GG	GA	AA		G	A			
SNP5	Affected	1628	491 (0.30)	786 (0.48)	351 (0.22)		1781 (0.54)	1501 (0.46)	0.070	1.09	0.440
(rs1116085)	Controls	1666	541 (0.32)	799 (0.48)	326 (0.20)	0.080	1868 (0.57)	1438 (0.43)	(0.196)	(0.99–1.21)	0.185

[a]The Cochran Armitage test.
[b]Fisher's exact test (two-sided).
[c]Permutation test (10 000 permutations).

Table 2 Haplotype comparisons between patients and control groups

Haplotype	*Frequency*		χ^2	*Individual* P *(uncorrected)*	*Global* P
	Patients	*Controls*			
SNP1–2–3–4–5					0.055
AGCGG	0.52	0.55	4.51	0.034	
GAGTA	0.26	0.24	3.09	0.079	
AAGTA	0.07	0.06	1.12	0.31	
AACGA	0.05	0.06	0.64	0.42	
AGCGA	0.05	0.05	1.36	0.24	
GACGA	0.02	0.01	0.41	0.52	
SNP1–2					0.023
AG	0.58	0.6	5.225	0.022	
GA[a]	0.29	0.27	4.390	0.036	
AA	0.13	0.13	0.002	0.96	

[a]The associated haplotype reported by Gerber *et al.*[8]

haplotype in the control group tended to be more frequently in the patient group ($P=0.079$). A global P-value for the 5 SNP haplotype was 0.055.

Gerber *et al.*[8] found associations of the G allele of SNP1 (CC21) and the A allele of SNP2 (CCS3) with schizophrenia by transmission disequilibrium test. In the present study, we found that the G allele of SNP1 and the A allele of SNP2 occurred more frequently in the patient group than in the control group ($P=0.003$, odds ratio (OR) = 1.15 for SNP1; $P=0.02$, one-sided, OR = 1.10 for SNP2). Gerber *et al.*[8] found that the most common haplotype was overtransmitted to patients in their US population. According to the data reported by Kinoshita *et al.*,[9] only two SNPs (SNP1 and SNP2 in the present study) are sufficient to distinguish the associated haplotype reported by Gerber *et al.*[8] from other haplotypes in the Japanese population. When haplotypes were constructed with SNP1 and SNP2, the haplotype that was the most common haplotype associated with schizophrenia in the US population was the second most common haplotype in our Japanese population. The second most common haplotype was more frequent in the patient group than in the control group ($P=0.036$, two-sided) in the present study (Table 2). Therefore, the present study replicates the allelic and haplotypic associations found in the US population described by Gerber *et al.*,[8] although the ORs of the haplotype were lower in the present study (OR = 1.12) than in the study by Gerber *et al.*[8] (OR = 1.23).

Kinoshita *et al.*[9] failed to replicate these associations. The OR of the G allele of SNP1 for schizophrenia

reported by Kinoshita *et al.*[9] was 1.11, which is similar to that (OR = 1.15) in the present study, although the ORs of the A allele of SNP2 and the second most common haplotype for schizophrenia were 1.0 in the Kinoshita *et al.*[9] study and 1.09 and 1.10 in the present study, respectively. Recently, Yamada *et al.*[7] reported a nominally significant association of SNP2 (CCS3, rs2461491) and a trend towards association of SNP1 (CC21, rs10108011) with schizophrenia in Japanese family based-association analysis.

The present study replicated the allelic and haplotypic associations of *PPP3CC* with schizophrenia. Thus, an association between genetic variations of *PPP3CC* and schizophrenia appears to exist in US and Japanese populations. However, the ORs of 1.10–1.15 observed in the present study indicate that the associations are weak and will be difficult to replicate without large sample sizes. Further studies are needed to evaluate wither alterations in calcineurin signaling contribute to the pathogenesis of schizophrenia.

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fMRI evidence for functional epistasis between COMT and RGS4

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COMT and RGS4 are promising candidate risk genes for schizophrenia[1] that impact on dopamine signaling[2–4] and on prefrontal function.[5,6] While, in general, convergent molecular pathways and neurophysiological effects implicate gene × gene interactions, there is growing evidence to support a specific direct interaction between risk alleles in COMT and RGS4. First, statistical epistasis between a number of putative schizophrenia risk genes, notably RGS4 and COMT, has been reported in risk for schizophrenia.[7] Second, a recent study in human postmortem dorsolateral prefrontal cortex demonstrated a significant correlation between both COMT val158met genotype and COMT enzyme activity and RGS4 mRNA levels, such that increased COMT enzyme activity (and Val allele load) predicted decreased RGS4 mRNA expression.[8] Using an fMRI working memory task in healthy subjects that robustly engages DLPFC in a manner sensitive to variation in both COMT and RGS4, we employed a moderated multiple regression approach to examine interactions between the COMT (rs4680 G/A(val158met)) and the RGS4 (rs951436 A/C ('Chowdari SNP4')),[9] SNPs most consistently associated with schizophrenia and with prefrontal activation. In line with earlier findings, we expected that the impact of genetic variation in RGS4 would be more pronounced on a COMT Val allele background, such that individuals with both RGS4 SNP4-A and COMT-Val would exhibit the most inefficient pattern of prefrontal cortical engagement (that is, greater prefrontal activation in the absence of performance differences).[10]

We studied 82 healthy Caucasian subjects using an N-back working memory task as described previously.[10] Subjects were genotyped for RGS4 and COMT[5,6] (COMT: 25 V/V, 43 V/M, 14 M/M; RGS4: 26 A/A, 36 A/C, 20 C/C). Since only two COMT Met/Met-RGS4 A/A subjects were available in this sample, V/M heterozygote and M/M homozygote subjects were combined. One-way analysis of variance (ANOVA) with five groups (COMT Val/Val, Met-carriers and RGS4 SNP4 A/A, A/C, C/C) revealed no significant between-group differences in performance (accuracy or reaction time), gender or age. Whole brain BOLD fMRI data were collected on a 3 T GE scanner (TE = 30 ms, TR = 2 s, flip angle = 90, FOV = 24 cm) while subjects performed an N-back working memory task (2-back and 0-back) as previously described.[10] A second level moderated multiple regression was used to map the main effects for

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ORIGINAL ARTICLE

Gap junction coding genes and schizophrenia: a genetic association study

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Abstract The aim of this study was to evaluate the association of genes that encode gap junction forming proteins and schizophrenia. Representative genetic candidates (Panx2 and Cx36) from two families of gap junction genes were selected for analysis. According to the present findings these genes represent both functional and positional candidates for schizophrenia. The sample was comprised of 381 schizophrenic patients, and the same number of matched controls was tested in this study in order to evaluate the possible influence of the aforementioned genes on the pathogenesis of schizophrenia. Four SNPs in the case of Panx2 and two SNPs in the case of Cx36 were selected for analysis. Allele-, genotype- and haplotype-wise association did not yield statistically significant results. These data do not suggest that Panx2 or Cx36 could increase the risk of schizophrenia in the Japanese population.

Keywords Gap junction · Panx2 · Cx36 · Schizophrenia · Genetic association · Case control · Gamma band oscillation

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Introduction

Schizophrenia is a severe mental disorder with a global morbid risk of approximately 1% and a strong genetic component in its etiology. The disease is characterized by abnormal perception, thought disturbances and impaired cognitive function (Sadock and Sadock 2005). One of the present views on the pathogenesis of schizophrenia is related to the disturbed connectivity of neuronal networks (Phillips and Silverstein 2003). The mechanism of the neuronal network depends on two types of synapses, namely, the usual chemical synapses and gap junctions (or electrical synapses) (Connors and Long 2004). A large body of evidence has shown that gap junctions have considerable influence on the stabilization and propagation of synchronized neuronal activity, especially in the beta (12–30 Hz) and gamma (30–80 Hz) frequency band of EEG (electroencephalogram) (LeBeau et al. 2003; Sohl et al. 2005). The aforementioned had been shown both in in vitro (Whittington et al. 1995; Szabadics et al. 2001; LeBeau et al. 2003; Mann et al. 2005; Traub et al. 2005) and in vivo studies, namely in experiments involving Cx36 (connexin 36) knock out mice (Hormuzdi et al. 2001; Buhl et al. 2003). Furthermore, gap junctions have the capability of bidirectional signal transmission and low-pass filter (LPF) characteristics that are markedly different from properties of chemical synapses. LPF as a term is used for describing the virtue of preferential transmission of sub-threshold potentials and low frequency stimuli that favor synchronous activity of neural networks (an extensive review is available at Sohl et al. 2005).

Two families of gap junction proteins have been described in humans: connexins and pannexins. The connexin family is divided into three subfamilies (alpha, beta and gamma) consisting of more than ten different proteins

(Rozental et al. 2000); however, Cx36 (subfamily gamma) is preferentially expressed in cell types of neural origin (Condorelli et al. 2000). Moreover, as has been mentioned before, several studies have indicated its role in the promotion of synchrony and power in a gamma frequency range of EEG (Hormuzdi et al. 2001; Buhl et al. 2003). The human pannexin (in a further text referred to as Panx) family consists of Panx1, Panx2 and Panx3. However, Panx2 is abundantly expressed in the brain and involved in forming gap junctions of principal cells or GABAergic interneurons (gamma amino butyric acid) (Bruzzone et al. 2003).

Regarding schizophrenia, several lines of evidence have showed decreased power and synchrony in the gamma frequency band of EEG during cognitive tasks in comparison to the nonaffected group (Spencer et al. 2003, 2004; Hong et al. 2004; Waberski et al. 2004). In that regard, the reported deficit in gamma band oscillations might be contributed by impaired synchronization of pyramidal cells owing to nonadequate perisomatic inhibition by paralbumin—expressed GABAergic interneurons, (Lewis et al. 2005).

Therefore, regarding gene expression and the reported role in the generation of gamma band oscillations, genes that encode Cx36 and Panx2 can be seen as functional candidates for schizophrenia. Moreover, chromosomal locations of both Cx36 (15q14) and Panx2 (22q13) are reported as linkage regions for schizophrenia (Meyer et al. 2002; Kenneth and Lindon 2005). For this reason, the aforementioned genes are positional candidates as well.

Methods

Sample population

The sample used in this research consisted of 381 schizophrenia patients (229 males and 151 females; mean age 50 ± 15.1 years) and 381 healthy controls (160 males and 221 females; mean age 40 ± 14.6 years). For analysis of Cx36, the total sample size consisted of 762 subjects and had been genotyped. On the other hand, the genetic association between schizophrenia and Panx2 was tested with a subset of the original sample comprised of 384 subjects (case control ratio 1:1). All the subjects were unrelated and of Japanese ethnicity. Subjects included in the case cohort met the DSM-IV criteria for schizophrenia with the consensus of two psychiatrists on the basis of an unstructured diagnostic interview and review of their medical records. Prior to inclusion in the control cohort subjects were screened on the basis of brief diagnostic interviews by an experienced psychiatrist. Subsequent to the study description, written informed consent was requested from each subject. This study was approved by the Ethics Committee at Nagoya University.

SNP selection

In order to test for genetic association either between the positional or functional candidate and phenotype of interest, we implemented a gene-based approach. This method implies inclusion of both the gene region and gene flanking regions in the association study (Neale and Sham 2004). In other words, it is important to understand the gene as a functional unity of coding and regulatory regions. Later on, by taking advantage of the observed linkage disequilibrium in the region of interest, it was possible to scale down the number of single nucleotide polymorphisms (SNPs) to be included in the association analysis by rejecting the redundant SNPs and picking only haplotype tagging SNPs in accordance with observed linkage disequilibrium (LD).

SNPs were selected from the hapmap database (release #21; phase II; July 2006, population: Japanese in Tokyo) (HapMap Consortium 2005), and the tagging SNP strategy was based on two criteria: first to exclude redundant SNPs from genotyping by taking advantage of the observed linkage disequlibrium in order to achieve 95% haplotype coverage and second to exclude SNPs with minor allele frequencies less than 10% (because of substantial loss of power due to sample size). According to the aforementioned criteria, from initial SNPs, four tagging SNPs for Panx2 (rs17284210, rs3817816, rs4838859 and rs7292533) and two tagging SNPs for Cx36 were selected (rs3743123 and rs752876).

None of the polymorphisms that had been tested in this study were functional; however, we can speculate on the possible impact on gene expression. Moreover, we tried to achieve good coverage of the gene and gene-adjacent regions. The rationale for the latter lies in the fact that tagging SNPs may be used as a proxy for detecting functional polymorphisms (i.e., LD between tagging SNP and unknown functional polymorphisms).

Genotyping methods

Genomic DNA was extracted from peripheral blood. The restriction fragment length polymorphism (RFLP) method was performed for genotyping rs17284210, rs3817816 and rs7292533. On the other hand, the allelic discrimination assay (Applied Biosystem Japan Ltd., Tokyo) was carried out for genotyping rs4838859, rs3743123 and rs752876. For each 384-well plate used for the allelic discrimination assay, three nontemplate controls were included. Details regarding primer design, cycle condition and restriction enzymes used for RFLP assay, as well as data on the probe design for allelic discrimination assay, are available upon request.

Statistical methods

The LD blocks were defined in accordance with the four gamete rule (Wang et al. 2002). Haplotype frequencies were estimated by the expectation-maximization algorithm. The aforementioned (LD block definition and haplotype frequency estimation) are functions implemented in the Haploview software v3.32 that was used in this study (Barrett et al. 2005). Genotype deviations from the Hardy–Weinberg equilibrium, allelic and genotypic associations were tested by chi-squared statistics. Log likelihood ratio tests for haplotypic association between schizophrenia and SNPs that are in linkage disequilibrium were carried out by the software Unphased v2.403 (Dudbridge 2003) with a permutation test for the calculation of empirical significance levels for differences between haplotype frequencies in case and control cohorts. The experimental alpha level was set to 0.05.

Power analysis was performed in accordance with the general power calculation model for chi-squared statistics. In brief, power is determined with respect to the degree of freedom and predefined alpha level of the study, after assuming the effect size (in accordance with Cohen's criteria). For the power calculation, Sample Power software v 2.0 (SPSS inc.) was used.

Results

HapMap data revealed a discreet linkage disequilibrium pattern in the region of Panx2. In detail, pairwise LD analysis of Panx2 polymorphisms showed one LD block that comprises several polymorphisms. However, only rs4838859 and rs7292533 were sufficient for 95% haplotype coverage. In spite of the fact that the remaining two polymorphisms (rs17284210 and rs3817816) are neither in linkage disequilibrium nor within a separate LD block, we included them in the study design in order to achieve satisfactory gene coverage for association analysis. Concerning Cx36, pairwise LD analysis showed one LD block that spreads throughout the gene region. Two SNPs (rs3743123 and rs752876) were sufficient in order to achieve 95% of haplotype coverage. The LD pattern of our data was in accordance with the LD pattern predicted by the HapMap database data set (data not shown).

Deviation from Hardy–Weinberg equilibrium was not observed. Allele-, genotype- or haplotype-wise analysis did not provide sufficient evidence for association between two genes that encode gap junction proteins and schizophrenia (see Table 1). Regarding Panx2, in case effect size is set to medium (in accordance with Cohen's criteria), the sample that was characterized in our research had approximately 80% power for detecting genetic association. However, in case the size effect had been set to small, the calculated power was 50%. On the other hand, regarding the Cx36 sample, the power was estimated to be more than 80% even if the effect size was set to small.

Discussion

The common disease-common variants hypothesis postulates that linkage disequilibrium should be detected by the

Table 1 Genotype distribution

Gene	SNP[a]	Block[b]	Cohort	Genotype[c]			*P* value	Allele[d]		*P* value	Haplotype *P* value
				M/M	M/m	m/m		M	m		
Cx 36	rs752876	I_{cx36}	Case	121	186	74	0.807	56.2	43.8	0.877	0.807
	G > A		Control	127	177	77		56.6	43.4		
	rs3743123		Case	138	176	67	0.803	59.3	40.7	0.958	
	C > T		Control	133	185	63		59.2	40.8		
Panx 2	rs17284210	/	Case	134	51	7	0.680	83.1	16.9	0.372	/
	T > G		Control	141	46	5		85.4	14.6		
	rs3817816	/	Case	90	79	23	0.987	67.4	32.6	0.878	/
	G > A		Control	89	79	24		66.9	33.1		
	rs4838859	I_{panx2}	Case	107	74	11	0.949	75.0	25.0	0.802	0.386
	A > G		Control	110	71	11		75.8	24.2		
	rs7292533		Case	84	94	14	0.344	68.2	31.8	0.539	
	T > C		Control	84	86	22		66.1	33.9		

[a] Upper value is dbSNP designation while lower value represents major > minor allele

[b] Dotted line represents LD block

[c] *M* Major allele, *m* minor allele in absolute numbers

[d] *M* Major allele, *m* minor allele in percents

haplotype association test if the risk haplotype was linked to variants (Chakravarti 1999). Therefore, regarding the Japanese population, the data presented in this article do not provide sufficient evidence for the involvement of the two gap junction coding genes in conferring that susceptibility.

Definition of phenotypes is vital for genetic association study; therefore, endophenotypes (being more specific than phenotypes) are thought to be important for this field (Gottesman and Gould 2003; Braff et al. 2007). We did not take advantage of endophenotypes in order to test for genetic association, and that might be a limitation of our study. However, here we have to mention that several studies showed gamma band oscillations, recorded in schizophrenic patients, that indeed have reduced power and synchrony in comparison to nonaffected subjects (Spencer et al. 2003, 2004). Therefore, rather that segregation of the sample in respect to the characteristic of gamma band oscillations, we implemented a crude phenotype approach (schizophrenia phenotype in toto) in the study design. Moreover, regarding Cx36, our data are in concordance with previous research regarding the aforementioned gene and schizophrenia (Meyer et al. 2002).

In conclusion, neither Panx2 nor Cx36 has influence on the pathogenesis of schizophrenia in the Japanese population. However, since this is the first study regarding Panx2 and schizophrenia, it might be interesting to explore the possible association in different population settings.

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Brief Research Communication

No Association Between Prostate Apoptosis Response 4 Gene (PAWR) in Schizophrenia and Mood Disorders in a Japanese Population

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Altered dopamine D2 receptor (D2R) is hypothesized to be a susceptibility factor for major psychosis. Recent studies showed that a new intracellular protein, prostate apoptosis response 4 (Par-4), plays a critical role in D2R signaling. We conducted a genetic association analysis between Par-4 gene (PAWR) and schizophrenia and mood disorders in a Japanese population (schizophrenia: 556 cases, bipolar disorder (BP): 150 cases, major depressive disorder (MDD): 312 cases and 466 controls). Applying the recommended 'gene-based' association analysis, we selected five tagging SNPs in PAWR from the HapMap database. No significant association was obtained found with schizophrenia or MDD or BP. We found a significant association of one tagging SNP with BP in a genotype-wise analysis ($P = 0.0396$); however, this might be resulted from type I error due to multiple testing ($P = 0.158$ after SNPSpD correction). Considering the size of our sample and strategy, our results suggest that the PAWR does not play a major role in schizophrenia or mood disorders in the Japanese population.

KEY WORDS: schizophrenia; mood disorders; Par-4; linkage disequilibrium; tagging SNP

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INTRODUCTION

An abnormal dopamine neurotransmission system is thought to contribute to the pathophysiology of psychiatric disorders such as schizophrenia and mood disorders, since the main target of most antipsychotics is dopamine D2 receptor (D2R) blockade. To date, several investigations have suggested that cellular mechanisms and signaling cascades related to D2R blockade may be associated with schizophrenia; for example AKT/Glycogen Synthase Kinase 3 (GSK3) in particular is an attractive candidate molecule for schizophrenia, since it has been reported from dopamine transporter (DAT) knock-out (KO) mice studies that the AKT/GSK3 cascade mediates dopamine-dependent behaviors through dopamine D2Rs [Beaulieu et al., 2004].

A more recent study showed that prostate apoptosis response 4 (Par-4) is also an attractive molecule from the viewpoint of psychiatric disorders [Park et al., 2005]. In this report, Par-4 was shown to interact with the D2R at the calmodulin bindings motif in the third cytoplasmic loop of the D2R, and formation of a Par-4/D2R complex was necessary to maintain an inhibitory tone on dopamine mediated cyclic AMP signaling generated by D2R in the low calcium condition [Park et al., 2005]. Moreover, Par-4 mutant mice showed depressive-like behaviors such as amotivation and anhedonia. These depressive-like symptoms of these mutant mice were reversed by antidepressants, indicating that Par-4 is also a good candidate molecule for mood disorders [Park et al., 2005].

The Par-4 gene (PAWR: OMIM *601936, 8 exons in this genomic region spanning 99.85 kb) is located on 12q21, which was shown to be a susceptibility region for schizophrenia [Wilcox et al., 2002], bipolar disorder (BP) [Craddock et al., 1994; Ewald et al., 2002], and major depressive disorder (MDD) [Craddock et al., 1994; Abkevich et al., 2003; McGuffin et al., 2005]. Considering all the above, Par-4 gene (PAWR) is a good candidate gene not only for schizophrenia but also for mood disorders (BP and MDD).

In this study, we aim to examine the genetic association between PAWR and schizophrenia or mood disorders in the Japanese population. To address this issue, we applied the recently recommended strategy of 'gene-based' association analysis [Neale and Sham, 2004]. We conducted a case-control association analysis using relatively large samples by selecting the 'tagging SNPs' from the HapMap database.

MATERIALS AND METHODS

Subjects

Five hundred and fifty-six patients with schizophrenia (289 males and 267 females; mean age ± standard deviation (SD) 42.84 ± 14.58 years, 45.13 ± 14.9 years), 312 patients with MDD (155 males and 157 females: 44.49 ± 14.09 years,

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50.43 ± 17.12 years), 150 patients with BP (76 males and 74 females: 93 patients with Bipolar I disorder and 57 patients with Bipolar II disorder: 46.58 ± 12.55 years, 45.05 ± 14.69 years) were recruited as case subjects, and a total of 466 healthy controls (243 males and 223 females: mean age ± SD 34.59 ± 12.58 years SD 36.16 ± 15.54 years) were recruited as control subjects. All subjects were unrelated with each other, ethnically Japanese, and lived in the central area of Japan.

The patients were diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of unstructured interviews and review of medical records. All healthy controls were also psychiatrically screened based on unstructured interviews. Subjects were free of past or present major or minor mental illness.

After description of the study, written informed consent was obtained from each subject. This study was approved by the Ethics Committee at Fujita Health University School of Medicine and Nagoya University Graduate School of Medicine.

SNP Selection and LD Evaluation

We first consulted the HapMap database (release #21/phase II, July 2006, www.hapmap.org, population: Japanese Tokyo: minor allele frequencies (MAFs) of more than 0.1) and included 32 SNPs covering PAWR (5′-flanking regions including 1,990 bp from the initial exon and 1,046 bp downstream (3′) from the last exon: HapMap database contig number chr12: 78483214...78590257) for an in silico 'tagging SNPs' selection. Then we applied the criterion of r^2 threshold greater than 0.8 in 'pair-wise tagging only' mode using the 'Tagger' program (Paul de Bakker, http://www/broad.mit.edu/mpg/tagger), an implement[Q1] of the HAPLOVIEW software program [Barrett et al., 2005], five 'tagging (tag) SNPs' (SNP1: rs2463169, SNP2: rs2400546, SNP3: rs17005769, SNP4: rs4842318, SNP5: rs7305141) were selected for the following association analysis.

SNP Genotyping

All SNPs were genotyped by TaqMan assay (Applied[Q2] Biosystems, CA). Detailed information is available on request.

Statistical Analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan).

Marker-trait association was evaluated by the χ^2 test (allele and genotype-wise analyses), and the log-likelihood test (haplotype-wise analysis, where the haplotype frequencies were estimated with the Expectation-Maximization algorithm) (SAS/Genetics, release 8.2). To control inflation of the type I error rate, we used a recently developed software program, SNPSpD, which can reflect the correlation of markers (LD) on corrected *P*-values [Nyholt, 2004].

Power calculation was performed using a statistical program prepared by Ohashi et al. [2001].

The significance level for all statistical tests was 0.05.

RESULT

Genotype frequencies of all SNPs were in HWE for each group (P values > 0.05). The result of LD structure from HapMap database can be seen in Supplementary Figure 1. Each LD structure of SCZ, BP, MDD, and control samples was almost same (data not shown).

No association was detected with schizophrenia or MDD in allele/genotype-wise analyses (Table I), or in haplotype-wise analysis (Global $P = 0.142$ in schizophrenia, 0.143 in MDD: Table II). Only SNP4 showed a significant association with BP in a genotype-wise analysis ($P = 0.0396$). Nevertheless, this significance may be a result of type I error due to multiple testing. We performed *P*-value correction by using SNPSpD program (the effective number of independent marker loci: 3.98; the experiment-wide significance threshold required to keep type I error rate at 0.05: 0.0126), and the haplotype-wise analysis showed no association with BP (Global $P = 0.158$).

Power analyses showed that the power was more than 80% when genotype relative risk at 1.30–1.33, 1.47–1.56, and 1.70–2.38 in schizophrenia, 1.34–1.37, 1.54–1.71, and 1.79–2.54 in MDD, 1.45–1.52, 1.79–2.11, and 2.10–3.16 in BP and under a multiplicative, dominant and recessive models of inheritance, respectively.

DISCUSSION

In this study, no association of PAWR with schizophrenia and mood disorders was found through genetic case-control study.

We included an explorative analysis of subjects divided by clinical diagnosis (except MDD) or sex, and no association was detected in any subgroup or in either sex (Supplementary Tables 1, 2, 3, 4, and 5). However, we assume that quantitative traits (e.g., negative symptoms for schizophrenia patients and severity of Hamilton Depression Scale for MDD patients) will be key features in assessing the genetic contribution of PAWR to schizophrenia or MDD, since Par-4 mutant mice showed depression-like symptoms.

Psychiatric disorders are widely known as complex diseases which are characterized by the contribution of multiple susceptibility genes and environmental factors. Even though our results did not support the contribution of PAWR to such disorders, novel candidate molecules or genes related to D2R signaling should be examined. In this regard, our colleagues examined the association of AKT1/GSK3β (and β-Arrestin2) with schizophrenia, and found that only AKT1 was associated with schizophrenia [Ikeda et al., 2004, 2005, 2007]. From these observations, we speculate that it may not be appropriate only to analyze the single-gene association for detecting susceptibility factors. Thus it will be necessary to account the gene–gene and gene-environmental interactions to obtain conclusive results.

A few points of caution about the present findings should be stressed. First, the lack of association may be due to biased samples, such as unmatched aged samples, or small sample size, especially BP samples. On the average level, the controls are much younger than the patients. This means that a number of young controls may go on to develop these disorders, the most likely MDD, since incidence of major depression is high as 5% or more. Second, we did not include a mutation scan to detect rare variants with functional effects. However, it is difficult to evaluate the association of such extremely rare variants (e.g., MAF < 0.01) from the viewpoint of power. Third, although Japanese population is considered to be homogeneous, small stratification may affect this negative finding. Replication study or family based association approach will be required for conclusive results.

Our results were robust in terms of study design, high powers of sample size, and conservative correction of multiple testing. Thus, we conclude that PAWR is unlikely to be a susceptibility gene for schizophrenia and mood disorders considering the common disease-common variant hypothesis. However, further investigations will be necessary for conclusive results.

TABLE I. tagSNPs and Association Analysis of PAWR

SNP ID	Phenotype	Position	MAF (%)	Genotype distribution[a]				*P*-value[b]			Corrected *P*-value (genotype)[c]
				N	M/M	M/m	m/m	HWE[d]	Allele	Genotype	
SNP1 rs2463169	Schizophrenia	0	0.300	556	277	224	55	0.329	0.872	0.517	
(G > A)	Bipolor disorder		0.273	150	80	58	12	0.744	0.317	0.502	
	Major depressive disorder		0.287	312	138	146	28	0.225	0.402	0.608	
	Controls		0.304	466	223	203	40	0.516			
SNP2 rs2400546	Schizophrenia	14682	0.240	556	328	189	39	0.106	0.786	0.474	
(A > T)	Bipolor disorder		0.233	150	90	50	10	0.403	0.953	0.717	
	Major depressive disorder		0.252	312	172	123	17	0.408	0.453	0.656	
	Controls		0.235	466	272	169	25	0.851			
SNP3 rs17005769	Schizophrenia	23921	0.248	556	321	194	41	0.125	0.254	0.414	
(G > A)	Bipolor disorder		0.317	150	68	69	13	0.441	0.121	0.205	
	Major depressive disorder		0.242	312	176	121	15	0.313	0.210	0.261	
	Controls		0.270	466	250	180	36	0.650			
SNP4 rs4842318	Schizophrenia	34881	0.293	556	282	222	52	0.388	0.822	0.161	
(C > T)	Bipolor disorder		0.243	150	89	49	12	0.167	0.128	**0.0396**	0.158
	Major depressive disorder		0.280	312	162	125	25	0.897	0.726	0.439	
	Controls		0.280	466	228	207	31	0.0777			
SNP5 rs7305141	Schizophrenia	63475	0.409	556	205	247	104	0.0556	0.986	0.237	
(A > G)	Bipolor disorder		0.463	150	39	83	28	0.168	0.0962	0.163	
	Major depressive disorder		0.365	312	122	152	38	0.372	0.0855	0.205	
	Controls		0.409	466	160	231	75	0.581			

[a]M, major allele; m, minor allele.
[b]Bold represents significant *P*-value.
[c]Calculated using SNPSpD software.
[d]Hardy–Weinberg equilibrium.

TABLE II. Individual and Global Haplotype-Wise Analyses of PAWR

Marker	Phenotype	Haplotype frequency	*P*-value
GAGTA	Schizophrenia	0.286	0.572
	Bipolor disorder	0.237	0.109
	Major depressive disorder	0.267	0.771
	Controls	0.273	
GAACG	Schizophrenia	0.231	0.078
	Bipolor disorder	0.302	0.229
	Major depressive disorder	0.231	0.095
	Controls	0.266	
ATGCA	Schizophrenia	0.226	0.910
	Bipolor disorder	0.229	0.994
	Major depressive disorder	0.240	0.577
	Controls	0.228	
GAGCG	Schizophrenia	0.149	0.247
	Bipolor disorder	0.154	0.423
	Major depressive disorder	0.125	0.744
	Controls	0.131	
AAGCA	Schizophrenia	0.0592	0.621
	Bipolor disorder	0.0399	0.0720
	Major depressive disorder	0.0681	0.752
	Controls	0.0644	
		Global *P*-value	
	Schizophrenia	0.142	
	Bipolor disorder	0.158	
	Major depressive disorder	0.143	

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Association analysis of AKT1 and schizophrenia in a UK case control sample

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Abstract

AKT1 (V-akt murine thyoma viral oncogene homolog 1) is involved in intracellular signalling pathways postulated as of aetiological importance in schizophrenia. Markers in the *AKT1* gene have also recently been associated with schizophrenia in two samples of European origin and in Japanese and Iranian samples. Aiming to replicate these findings, we examined ten SNPs spanning *AKT1* in a UK case-control sample (schizophrenia cases $n=673$, controls $n=716$). These included all SNPs previously reported to be associated in European, Japanese and Iranian samples, alone or in haplotypes, as well as additional markers defined by the Haploview Tagger program (pair-wise tagging, minimum $r^2=0.8$, minor allele frequency=0.02). We found no association with single markers (min $p=0.17$). We found weak evidence for association ($p=0.04$) with a four marker haplotype reported as significant in the original positive European sample of Emamian et al. [Emamian, E.S., Hall, D., Birnbaum, M.J., Karayiorgou, M., Gogos, J.A., 2004. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. Nat. Genet. 36, 131–137] and also an overlapping three marker haplotype ($p=0.016$) that had previously been reported as significant in a Japanese sample. Nominal p-values for these haplotypes did not survive correction for multiple testing. Our study provides at best weak support for the hypothesis that *AKT1* is a susceptibility gene for schizophrenia. Examination of our own data and those of other groups leads us to conclude that overall, the evidence for association of *AKT1* as a susceptibility gene for schizophrenia is weakly positive, but not yet convincing.

Keywords: AKT1; Schizophrenia; Association; Candidate gene

1. Introduction

Emamian et al. (2004) proposed that alterations in brain protein kinase activity contribute to the aetiology of schizophrenia. In pursuit of this hypothesis, they examined the abundance of seven protein kinases in lymphoblast cell lines. Reduced AKT1 expression was found in cell lines derived from schizophrenic patients compared to controls, a finding subsequently confirmed in *post-mortem* frontal cortex and hippocampus. Moreover, they also found reduced phosphorylation of GSKβ3, a substrate of AKT1. These data provide a

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plausible case that AKT1 might be involved in the pathophysiology of schizophrenia, a hypothesis whose aetiological relevance they explored by genetic association using 5 SNPs spanning the gene (see Tables 1–3 for SNP nomenclature) in 268 US families of North European origin containing one or more individuals with schizophrenia. The initial evidence for association was weak but one marker, (SNP3), yielded evidence for association ($p=0.05$, uncorrected) as did a number of haplotypes (minimum $p=0.04$, corrected) each of which shared alleles T and C at SNPs 2 and 3 (Emamian et al., 2004), (referenced hereafter as the core haplotype). The core haplotype was also associated with reduced AKT1 protein expression in 20 control lymphoblast cell lines. Follow-up studies in three independent Japanese samples gave mixed results. Two studies consisted of over 500 cases and over 400 controls. The first (Ohtsuki et al., 2004) found no association (allelic or haplotypic) while the second (Ikeda et al., 2004) reported weak evidence for association with a different variant and different haplotype to that of Emamian et al. (2004) (Table 2), with allele C of the core being carried in haplotypes that were both over and underrepresented in cases. A third study in a Japanese sample of 124 families found no association (allelic or haplotypic) (Ide et al., 2006). Schwab et al. (2005) found significant association with 3 of 7 SNPs tested in *AKT1* in 79 sib pair families of German origin. The associated SNPs included SNP3, $p=0.027$, which was nominally significant in the Emamian study as well as two other SNPs, with the strongest result (SNP2a, rs10149779, $p=0.002$) remaining significant after correction for multiple testing ($p=0.014$). The most significant haplotype from the Emamian study (SNP2/SNP3/SNP4, TCG, Table 2) was also significantly over-represented in cases as was the TTA haplotype (formed by the same SNPs), which had been under-transmitted to cases in the study of Emamian et al. (2004) and which does not carry the core TC haplotype. Several other haplotypes created by various permutations of markers were also significantly over-transmitted with illness, with the strongest evidence coming from a haplotype derived from SNP1/SNP2a/SNP3 ($p=0.0013$ corrected for multiple testing), Table 2. For all haplotypes in which SNP2 was included, the over-transmitted haplotype carried Emamian's core T allele at SNP2 but the finding of the earlier study was not precisely recapitulated since haplotypes carrying either C or T at core SNP3 were significantly over-transmitted.

Further studies have been less supportive (Bajestan et al., 2006; Liu et al., 2006). The 5 SNPs genotyped by Emamian et al. (2004) were genotyped in 218 families from Taiwan (Liu et al., 2006) with no significant association from either single markers or haplotypes. The same SNPs were also typed in an Iranian case control sample, (schizophrenia cases $n=321$, controls $n=383$) (Bajestan et al., 2006). Again, neither the SNPs nor the

Table 1
Results: single markers

SNP ID	Dist to next SNP (bp)	Base change allele 1/2	Sample sized	Allele 1 count (freq)	Allele 2 count (freq)	*p*-value (1df)
rs3803300 (SNP1)	4816	G/A	Case ($N=660$)	1194 (0.90)	126 (0.10)	0.40
			Control ($N=707$)	1292 (0.91)	122 (0.09)	
rs2498784 (SNP1a)	5229	T/C	Case ($N=658$)	102 (0.08)	1214 (0.92)	0.93
			Control ($N=712$)	109 (0.08)	1315 (0.92)	
rs1130214 (SNP2)	8648	T/G	Case ($N=586$)	361 (0.31)	811 (0.69)	0.73
			Control ($N=660$)	415 (0.31)	905 (0.69)	
rs10149779 (SNP2a)	4400	A/G	Case ($N=658$)	398 (0.30)	918 (0.70)	0.69
			Control ($N=711$)	440 (0.31)	982 (0.69)	
rs2494738	279	A/G	Case ($N=662$)	109 (0.08)	1215 (0.92)	0.68
			Control ($N=705$)	110 (0.08)	1300 (0.92)	
rs3730358 (SNP3)	6513	C/T	Case ($N=608$)	1043 (0.86)	173 (0.14)	0.30
			Control ($N=679$)	1145 (0.84)	213 (0.16)	
rs2498799 (SNP4)	702	G/A	Case ($N=592$)	918 (0.78)	266 (0.22)	0.17
			Control ($N=659$)	991 (0.75)	327 (0.25)	
rs2494732 (SNP5)	46	T/C	Case ($N=588$)	652 (0.55)	524 (0.45)	0.80
			Control ($N=663$)	742 (0.56)	584 (0.44)	
rs3803304	6051	C/G	Case ($N=653$)	327 (0.25)	979 (0.75)	0.56
			Control ($N=707$)	368 (0.26)	1046 (0.74)	
rs2498804 (SNP A)	–	G/T	Case ($N=660$)	911 (0.69)	409 (0.31)	0.46
			Control ($N=709$)	960 (0.68)	458 (0.32)	

Allele counts, frequencies and *p*-values across *AKT1* locus. SNP ID includes both rs no. and ID used in Emamian et al. (2004), Ikeda et al. (2004) and Schwab et al. (2005).

Table 2
Comparison of associated haplotypes

Study	Population	rs3803300 (SNP1)	rs2498784 (SNP1a)	rs1130214 (SNP2)	rs10149779 (SNP2a)	rs2494738	rs3730358 (SNP3)	rs2498799 (SNP4)	rs2494732 (SNP5)	rs3803304	rs2498804 (SNPA)	SCZ	CON	*P*-value
Emamian et al.	US			T			C*	G*				–	0.15	0.0006
Schwab et al.	German			T			C*	G*				0.17	0.10	0.023
This study	UK			T			C	G				0.19	0.17	0.37
Schwab et al.	German	G*			T		C*					0.17	0.09	0.0013
This study	UK	G			T		C					0.18	0.16	0.51
Emamian et al.	US			T			C*	G*	G			–	–	0.004
This study	UK			T			C*	G*	G			0.13	0.10	0.04
Schwab et al.	German			T			C	G	G			0.10	0.07	0.11[a]
Ikeda et al.	Japanese			T			C	G	G			0.02	0.01	0.18[a]
Ikeda et al.	Japanese						C*	G*	G			0.32	0.27	0.014
This study	UK						C*	G*	G			0.22	0.17	0.016
Bajestan et al.	Iranian	A		G*			C	A	G			0.07	0.03	0.004
This study	UK	A		G			C	A	G			0.05	0.05	0.73
This study	UK	A					C*	G*				0.04	0.02	0.006
Ikeda et al.	Japanese						C*	G*	A			0.17	0.24	0.0001
This study	UK						C	G	A			0.55	0.56	0.55

Comparison of the most significant *p*-values from current studies reporting positive association with *AKT1* and schizophrenia. Significant haplotypes are marked in grey.
[a]Personal communications, *Ancestral allele in NCBI entrez SNP.

haplotypes from the Emamian study were associated. However, a novel five marker haplotype comprised of SNPs1-5, showed some evidence for association (global $p=0.05$ uncorrected) with haplotype AGCAG being more frequent in cases compared to controls (uncorrected $p=0.004$, Bonferroni corrected, $p=0.03$, case freq 0.068, control freq 0.034). Given the diverse range of ethnicities studied so far, lack of consistency of the patterns of association between studies is potentially explicable in terms of population differences in LD and modest power to detect weak genetic effects. Moreover, in the light of partial replication of the original findings at the level of a specific haplotype in the only other European origin sample so far reported, *AKT1* is clearly worth further investigation in other samples of broadly similar ethnicity.

We set out to investigate *AKT1* in schizophrenia using a moderately large UK based case control sample under the following strategies. We genotyped SNPs 1–5 from Emamian et al. (2004), and additional markers

Table 3
LD data for control sample

	rs3803300 (SNP1)	rs2498784 (SNP1a)	rs1130214 (SNP2)	rs10149779 (SNP2a)	rs2494738	rs3730358 (SNP3)	rs2498799 (SNP4)	rs2494732 (SNP5)	rs3803304	rs2498804 (SNPA)
rs3803300 (SNP1)	x	0.86	0.01	0.01	0.46	0.01	0.11	0.07	0.00	0.12
rs2498784 (SNP1a)	0.98	x	0.02	0.02	0.38	0.00	0.11	0.06	0.00	0.10
rs1130214 (SNP2)	0.36	0.78	x	0.95	0.00	0.25	0.06	0.21	0.11	0.06
rs10149779 (SNP2a)	0.41	0.81	0.99	x	0.01	0.26	0.06	0.20	0.12	0.07
rs2494738	0.71	0.62	0.30	0.36	x	0.02	0.12	0.09	0.00	0.16
rs3730358 (SNP3)	0.63	0.55	0.80	0.80	1	x	0.40	0.22	0.49	0.37
rs2498799 (SNP4)	0.63	0.70	0.29	0.30	0.71	0.85	x	0.43	0.40	0.69
rs2494732 (SNP5)	0.77	0.79	0.60	0.59	0.92	0.98	1	x	0.44	0.59
rs3803304	0.05	0.18	0.37	0.38	0.14	0.96	0.65	1	x	0.69
rs2498804 (SNPA)	0.79	0.76	0.25	0.27	0.95	0.96	0.99	1	0.97	x

LD data for control sample. D' is below diagonal and r^2 is above diagonal.

reported by others, SNP1a, SNP2a, (Schwab et al., 2005) and SNPA (Ikeda et al., 2004). We specifically tested all significant associated haplotypes reported by Emamian et al. (2004), (n=7), Ikeda et al. (2004), (n=9), Schwab et al. (2005), (n=23) and the Iranian 5 marker haplotype (Bajestan et al., 2006), (a total of 30 tests), although our primary hypothesis concerned the European origin haplotypes (n=28). Additionally, we derived tagged SNPs across the *AKT1* locus after genotyping all the above markers in the CEU panel used by the HapMap project and combining those data with all additional markers available in the HapMap (version 1.65) and performed two and three marker haplotype analyses for all marker combinations.

2. Materials and methods

2.1. Subjects

All case-control subjects used in this study were unrelated Caucasians born in the UK or Ireland. All cases met DSM-IV criteria for schizophrenia. Consensus diagnoses were made by two raters from all available information following a semi-structured interview, SCAN or PSE (Wing et al., 1974, 1990), and examination of case notes. The cases consisted of 456 males and 217 females, average age at collection 44.5 years ±14.6, whilst the controls consisted of 482 males and 234 females, average age at collection 41.5 years ±11.5 years. Control individuals were group matched to cases for age, sex, and ethnicity from more than 1400 blood donors recruited from the National Blood Transfusion Service. Individuals on medication are not allowed to donate blood in the UK nor are they remunerated even for expenses. Thus unlike in some countries, donating blood in the UK is entirely an altruistic process that does not tend to enrich for indigents, or people with substance abuse or psychosis. Donors were not screened for the absence of psychiatric illness, as this does not affect the power when a disease has the population prevalence of schizophrenia (Owen et al., 1997). Multicentre and Local Research Ethics Committee approval was obtained, and all subjects, both cases and controls, gave written informed consent to participate. We previously found no evidence for population stratification within the samples based on the distribution of p-values obtained from genotyping pooled samples for >300 SNPs (Williams et al., 2005a). We also tested for evidence of substructure in approximately one-third of our sample with STRUCTURE (Pritchard et al., 2000) by using 97 SNPs scattered across the genome and 1000 SNPs targeted to regions on chromosomes 10 and 22 (Georgieva et al., 2006).

Sample power was estimated to be 80% for the "core TC haplotype" given our observed frequency, an OR of 1.3, alpha=0.05 and 79% for the associated TCG haplotype in Table 2, under the same parameters. For rs3730358 (associated in both Emamian et al., 2004 and Schwab et al., 2005), we estimated power to be 73% given an OR of 1.3, alpha=0.05.

2.2. SNP selection

We initially selected for genotyping, SNPs 1–5 from Emamian et al. (2004), (rs3803300, rs1130214, rs3730358, rs2498799, rs2494732 respectively), two additional SNPs from Schwab et al. (2005), (rs2498784 and rs10149779, SNP1a and SNP2a respectively), and 1 additional SNP from Ikeda et al. (2004), (rs2498804, SNPA), in order to be able to test the specific marker and haplotype hypotheses generated by those studies. All SNPs were optimised on the same CEPH DNA samples used in the international HapMap project for purposes of both error checking (all genotypes were checked against HapMap data for concordance) and also for tag SNP selection. We used our CEPH data and all other available CEPH data from the HapMap release 16C.1 June 2005 (Generic genome browser version 1.65) across the *AKT1 locus* from UCSC May2004 chr14:104304140–104341530 (including 8.4 kb sequence upstream and 2.6 kb sequence downstream of *AKT1*) and performed pairwise analysis with TAGGER as implemented in Haploview (Barrett et al., 2005) using settings r^2>0.8, minimum MAF 2%. This suggested as additional tagging SNPs rs2494738 and rs3803304, none of which have been genotyped in previous *AKT1* association studies (Emamian et al., 2004; Ohtsuki et al., 2004; Ikeda et al., 2004; Schwab et al., 2005; Liu et al., 2006; Bajestan et al., 2006; Ide et al., 2006).

2.3. Genotyping

8/10 SNPs were genotyped using the Sequenom MassARRAY™ system as per the manufacturer's instructions with either hME or iplex chemistries. SNPs 1a and 2a were genotyped with allele-specific PCR using the Amplifluor system (Myakishev et al., 2001; Hawskins et al., 2002).

Assay design and PCR conditions are available on request. All assays used to type the full association sample were optimised initially by genotyping DNA from 30 CEPH parent–offspring trios from 21 families (Utah residents with ancestry from northern and western Europe), as detailed in the international HapMap project