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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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REFERENCES

Abkevich V, Camp NJ, Hensel CH, Neff CD, Russell DL, Hughes DC, Plenk AM, Lowry MR, Richards RL, Carter C, Frech GC, Stone S, Rowe K, Chau CA, Cortado K, Hunt A, Luce K, O'Neil G, Poarch J, Potter J, Poulsen GH, Saxton H, Bernat-Sestak M, Thompson V, Gutin A, Skolnick MH, Shattuck D, Cannon-Albright L. 2003. Predisposition locus for major depression at chromosome 12q22-12q23.2. Am J Hum Genet 73:1271–1281.

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265.

Beaulieu JM, Sotnikova TD, Yao WD, Kockeritz L, Woodgett JR, Gainetdinov RR, Caron MG. 2004. Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. Proc Natl Acad Sci USA 101:5099–5104.

Craddock N, McGuffin P, Owen M. 1994. Darier's disease cosegregating with affective disorder. Br J Psychiatry 165:272.

Ewald H, Flint T, Kruse TA, Mors O. 2002. A genome-wide scan shows significant linkage between bipolar disorder and chromosome 12q24.3 and suggestive linkage to chromosomes 1p22-21, 4p16, 6q14-22, 10q26 and 16p13.3. Mol Psychiatry 7:734–744.

Ikeda M, Iwata N, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshita Y, Inada T, Ozaki N. 2004. Association of AKT1 with schizophrenia confirmed in a Japanese population. Biol Psychiatry 56:698–700.

Ikeda M, Iwata N, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshita Y, Ozaki N. 2005. No association of GSK3beta gene (GSK3B) with Japanese schizophrenia. Am J Med Genet Part B 134B:90–92.

Ikeda M, Ozaki N, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshita Y, Kishi T, Sekine Y, Iyo M, Harano M, Komiyama T, Yamada M, Sora I, Ujike H, Inada T, Iwata N. 2007. Possible association of beta-arrestin 2 gene with methamphetamine use disorder, but not schizophrenia. Genes Brain Behav 6:107–112.

McGuffin P, Knight J, Breen G, Brewster S, Boyd PR, Craddock N, Gill M, Korszun A, Maier W, Middleton L, Mors O, Owen MJ, Perry J, Preisig M, Reich T, Rice J, Rietschel M, Jones L, Sham P, Farmer AE. 2005. Whole genome linkage scan of recurrent depressive disorder from the depression network study. Hum Mol Genet 14:3337–3345.

Neale BM, Sham PC. 2004. The future of association studies: Gene-based analysis and replication. Am J Hum Genet 75:353–362.

Nyholt DR. 2004. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 74:765–769.

Ohashi J, Yamamoto S, Tsuchiya N, Hatta Y, Komata T, Matsushita M, Tokunaga K. 2001. Comparison of statistical power between 2 * 2 allele frequency and allele positivity tables in case-control studies of complex disease genes. Ann Hum Genet 65:197–206.

Park SK, Nguyen MD, Fischer A, Luke MP, Affar el B, Dieffenbach PB, Tseng HC, Shi Y, Tsai LH. 2005. Par-4 links dopamine signaling and depression. Cell 122:275–287.

Wilcox MA, Faraone SV, Su J, Van Eerdewegh P, Tsuang MT. 2002. Genome scan of three quantitative traits in schizophrenia pedigrees. Biol Psychiatry 52:847–885.

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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

(The International HapMap Consortium, 2005). We re-genotyped 46 of these samples along with the case control sample to provide a measure of genotyping accuracy. All genotypes were called blind to sample identity and affected status.

2.4. Statistical analysis

Tests of genotypic and allelic association were performed using contingency tables. Haplotype analyses were performed using the EM algorithm and a permutation test as implemented in program EH plus (Zhao et al., 2000) for global significance. Association of specific haplotypes was estimated with Cocaphase (Dudbridge, 2003). LD values were calculated using the ldmax program within the GOLD software (Abecasis and Cookson, 2000).

3. Results

Genotype data for SNP2, rs2494738, rs3803304 and SNPA from our assays in the same 90 CEPH DNA samples used in the International HapMap Project were 100% concordant with HapMap data. 100% concordance was also achieved between genotype data of 46 CEPH DNA samples typed in our initial assay optimisation stage and the same samples contained within our case control sample set for all 10 SNPs.

Genotype data were in Hardy Weinberg equilibrium for both cases and controls for all SNPs. No significant differences between cases and controls were observed for any single markers by allele (Table 1) or genotype (data not shown).

We specifically tested those marker combinations reported to yield the most significantly associated haplotypes by Emamian et al. (2004) including the core haplotype, (SNP2/SNP3, TC), those of Schwab et al. (2005), (SNP1/SNP2a/SNP3, GTC) and Ikeda et al. (2004), (SNP3/SNP4/SNP5, CGG and CGA as well as seven other overlapping haplotypes also significantly associated in the Japanese sample). Table 2 summarizes the most significant haplotypes with the ancestral alleles marked as *. Marker combination SNP2/SNP3/SNP4 which gave the most significant results in the Emamian study, gave a global p-value of 0.08 in our sample. However, the TCG haplotype for this marker combination which gave nominal significance in the initial Emamian study ($p=0.0006$) and also in that of Schwab et al. (2005), ($p=0.02$) was not significantly associated in our sample, $p=0.37$, although a non significant trend was observed in the previously reported direction (Table 2). Our case and control frequencies for this haplotype were 0.19 and 0.17 respectively, compared to 0.17 and 0.10 in the German sample (Schwab et al., 2005) and 0.15 in the US sample of European origin of Emamian et al. (2004).

Global haplotype analysis of markers (SNP2/SNP3) forming the core two-marker haplotype of Emamian et al. (2004) revealed no significant evidence for association (global $p=0.09$) nor did specific analysis of the TC phased core haplotype ($p=0.41$). However, a specific phased 4 marker haplotype (SNP2/SNP3/SNP4/SNP5, TCGG, Table 2) which was also significant in the Emamian study, $p=0.004$, was associated in our sample, $p=0.04$ (case freq=0.13, control freq=0.10). The same haplotype was not significantly associated in either the German (Schwab et al., 2005) or in the Japanese (Ikeda et al., 2004) samples, ($p=0.11$ and 0.18 respectively), (personal communications).

The most significant haplotype of (SNP1/SNP2a/SNP3 GTC, Table 2) reported by Schwab et al. (2005) was not associated in our sample, ($p=0.51$), although the trend with frequencies of 0.18 and 0.16 in our cases and controls respectively, compared to 0.17 and 0.09 in the German sample was in the same direction.

Global haplotype analysis of SNP3/SNP4/SNP5 which was the most significant haplotype in the Japanese study of Ikeda et al. (2004) was significantly associated (uncorrected $p=0.04$) in our sample. The largest difference in haplotype frequency for this combination was 5% for the haplotype CGG (Table 2, $p=0.016$). The same haplotype was also significantly associated in the same direction in the Japanese sample (Ikeda et al., 2004), $p=0.014$, although frequencies in the samples are different (CGG=0.27 in Japanese controls vs. 0.17 in Cardiff controls). The most significant haplotype associated in the Japanese sample was SNP3/SNP4/SNP5 CGA, ($p=0.0001$), (Ikeda et al., 2004). This was not significantly associated in our sample. The haplotype frequencies in our sample and in that of Ikeda et al. (2004) are substantially different (Table 2). Ikeda et al. (2004) also reported 7 overlapping haplotypes with individual p-values less than 0.05. None of these was significantly associated in our sample.

Although we selected the markers predicated on single locus (i.e. pairwise tagging), in order to try to capture the effect of unknown variants that are not represented in HapMap, we undertook 2 and 3 marker haplotype analysis across all the markers including those additional SNPs recommended by our tagging procedure. We obtained evidence for association for haplotypes of SNP1/SNP3/SNP4, global $p=0.04$, which overlaps physically with the most significant haplotypes reported by Schwab et al. (2005) and Emamian et al. (2004). On closer inspection, the effect came from two haplotypes with frequencies of

less than 5%, (GTG case=0.009, control=0.018, $p=0.06$ and ACG, case=0.043, control=0.023, $p=0.006$). Allele C of SNP3 is common to our significant haplotype (SNP1/SNP3/SNP4, ACG) and the most significant haplotypes of Emamian et al. (2004) and Schwab et al. (2005), whilst allele G of SNP4 is common to our SNP1/SNP3/SNP4, ACG haplotype and the SNP2/SNP3/SNP4, TCG haplotype of Emamian et al. (2004) and Schwab et al. (2005).

4. Discussion

Following the initial report (Emamian et al., 2004) and mixed replication evidence (Ohtsuki et al., 2004; Ikeda et al., 2004; Ide et al., 2006; Schwab et al., 2005; Liu et al., 2006; Bajestan et al., 2006) we sought to provide further evidence for association between schizophrenia and polymorphisms in *AKT1*. The question of when the evidence for association between disease and gene is convincing is a vexing one for several reasons. Ideally, such evidence would come from repeated demonstration of a directional association (even if not significant) between a disorder and a specified allele such that pooled or meta-analyses demonstrates a clear highly significant directional effect. However, when based upon indirect association, replication of specified alleles may not be easily obtained due to a mixture of population differences in allelic heterogeneity at the locus, patterns of LD, allele frequencies, phenotypic variation relevant to the associated allele, or exposure to environmental variables with which a risk allele interacts (O'Donovan and Owen, 1999). Moreover, mathematical analyses show that where the true effect size of a susceptibility allele is weak, opposite alleles may be genuinely associated with disease even in populations with similar LD measures, allele frequencies, and identical effect sizes at the functional locus (Moskvina and O'Donovan, in press). Given the above, while association to the same allele across studies should at least be sought, it cannot be a prerequisite for considering a study as supportive of association between disease and a gene. Instead, we believe it is legitimate to view association to any allele or haplotype that both survives honest appropriate correction for multiple testing and is based on a well-designed quality-controlled study as significant evidence for replication at the gene-level. When multiple studies meet this criterion, as we consider to be the case for example for dysbindin (Williams et al., 2005b), then in our view, the evidence can be considered convincing.

Our single marker data for AKT1 provide no evidence for association with schizophrenia, but haplotype analysis showed some trends similar to the existing data, albeit, none that remain significantly associated in the context of multiple testing. When associated haplotypes from all of these studies are aligned (Table 2), it is apparent that the most significant risk haplotypes across studies overlap, making the correction for multiple testing over conservative. (Of the p-values reported in Table 2, the most significant haplotype of Schwab et al. (2005) is already corrected for multiple testing by simulation (Becker and Knapp, 2004). The most significant haplotype in the Emamian study ($p=0.006$) is uncorrected, but remains significant after adjustment for the comparisons made in the study, ($p<0.04$).

In Table 2 where we show the most significant haplotype reported from each study and compare these specific haplotypes across the published data and with our own data, the SNP3/4, alleles CG combination occurs in 6/7 of the significant haplotypes. This trend also extends to the Japanese sample (Ikeda et al., 2004), but not in the Iranian sample (Bajestan et al., 2006) where the only significant haplotype contains the A allele at SNP 4. Also, when all ethnicities are considered together, a trend for a longer overlap is observed with SNPs 3/4/5, CGG in the significant risk haplotypes of both this, the initial positive and the Japanese study, (4/10 significant haplotypes). However, given that alleles C and G are respectively the major alleles at SNP3 and SNP4, and are present on numerically more haplotypes than SNP3 allele T, this may simply be chance rather than a reflection of a genuine pattern in the data. Nevertheless, similar directions of effect were observed in our sample for the haplotypes most significantly over-represented in samples of European origin. Further confidence could be achieved if the four marker haplotype (SNP2/SNP3/SNP4/SNP5, TCGG) which was associated in the original positive study and in our own data was also significant in the German study and Japanese samples, particularly since (SNP3/SNP4/SNP5, CGG) was also significant in the Japanese sample. However, the TCGG haplotype was not significantly associated in the German sample, $p=0.11$ (personal communication) although the trend was in the same direction, and the same haplotype was of low frequency in the Japanese sample of Ikeda et al. (2004), (personal communication) and was not significant in the Japanese sample of Ohtsuki et al. (2004), (personal communication).

Our haplotype frequencies were broadly similar to those other samples of European, origin (Emamian et al., 2004; Schwab et al., 2005), although when compared to the German sample (Schwab et al., 2005), both our case and control frequencies were more similar to the German cases than to the German controls (Table 2). Comparison of LD patterns across studies showed D'

values for our sample to be very similar to those of Emamian et al. (2004) and also to those of Schwab et al. (2005) and the CEPH sample (Table 3). Schwab et al. (2005) also suggested increased recombination in the region between SNP2a and SNP3 based on a decrease in D' in this region compared to neighbouring regions. By genotyping all markers in the same CEPH individuals used in the international HapMap project, our data agree with this finding (data not shown).

4.1. Conclusions

In a bid to replicate association of *AKT1* with schizophrenia, we genotyped those polymorphisms that provided evidence for association in samples of both European and Japanese origin and also undertook a pairwise analysis across the locus in a large UK based case control sample. None of our findings survive correction for even modest degrees of multiple testing, and therefore we must conclude that our study does not provide robust support for the hypothesis. However, in our sample, we find (uncorrected) support for a phased 4 marker haplotype that was significantly associated in the original positive association study (Emamian et al., 2004) and which also shows non-significant trends in the same direction in the only other sample of white European origin (Schwab et al., 2005), (p=0.11, 2-tailed) and, notwithstanding a very low frequency, in the positive Japanese study (Ikeda et al., 2004), (p= 0.18). This phased haplotype also shares 3 alleles with a significant phased 3 marker haplotype in a Japanese sample (Ikeda et al., 2004). Thus, while our study does not provide strong support, the current evidence for association between *AKT1* across different studies remains intriguing, and worthy of further investigation.

5. Contributors

NN, HW, SD, LC, TP performed laboratory assays. NN performed the data-analysis and drafted the manuscript. VM and RS advised on data-analysis. NW participated in the design of the study. IN was responsible for data-management. MI and NI provided haplotypic data and analysis from an independent sample. MOD and MJO participated in the design of the study, interpretation of the data, and drafting of the manuscript. All authors read and approved the final manuscript.

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7. Conflicts of interest

The author(s) declare that they have no conflicts of interest.

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References

Abecasis, G.R., Cookson, W.O., 2000. GOLD-graphical overview of linkage disequilibrium. Bioinformatics 16, 182–183.

Bajestan, S.N., Sabouri, A.H., Nakamura, M., Takashima, H., Keikhaee, M.R., Behdani, F., et al., 2006. Association of AKT1 haplotype with the risk of schizophrenia in Iranian population. Am. J. Med. Genet. B Neuropsychiatr. Genet. 141, 383–386.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265.

Becker, T., Knapp, M., 2004. A powerful strategy to account for multiple testing in the context of haplotype analysis. Am. J. Hum. Genet. 75, 561–570.

Dudbridge, F., 2003. Pedigree disequilibrium tests for multilocus haplotypes. Genet. Epidemiol. 25, 115–121.

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.

Georgieva, L., Moskvina, V., Peirce, T., Norton, N., Bray, N.J., Jones, L., et al., 2006. Convergent evidence that oligodendrocyte lineage transcription factor 2 (OLIG2) and interacting genes influence susceptibility to schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 103, 12469–12474.

Hawskins, J.R., Khripin, Y., Valdes, A.M., Weaver, T.A., 2002. Miniaturized sealed-tubes allele-specific PCR. Human Mutat. 19, 543–553.

Ide, M., Ohnishi, T., Murayama, M., Matsumoto, I., Yamada, K., Iwayama, Y., 2006. Failure to support a genetic contribution of AKT1 polymorphisms and altered AKT signaling in schizophrenia. J. Neurochem. 99, 277–287.

Ikeda, M., Iwata, N., Suzuki, T., Kitajima, T., Yamanouchi, Y., Kinoshita, Y., et al., 2004. Association of AKT1 with schizophrenia confirmed in a Japanese population. Biol. Psychiatry 56, 698–700.

Liu, Y.L., Fann, C.S., Liu, C.M., Wu, J.Y., Hung, S.I., Chan, H.Y., et al., 2006. Absence of significant associations between four AKT1 SNP markers and schizophrenia in the Taiwanese population. Psychiatr. Genet. 16, 39–41.

Moskvina, V., O'Donovan, M.C., in press. Detailed analysis of the relative power of direct and indirect association studies and the implications for their interpretation. Hum. Hered.

Myakishev, M.V., Khripin, Y., Hu, S., Hamer, D.H., 2001. High-throughput SNP genotyping by allele-specific PCR with universal energy-transfer-labeled primers. Genome Res. 11, 163–169.

O'Donovan, M.C., Owen, M.J., 1999. Candidate-gene association studies of schizophrenia. Am. J. Hum. Genet. 65, 587–592.

Ohtsuki, T., Inada, T., Arinami, T., 2004. Failure to confirm association between AKT1 haplotype and schizophrenia in a Japanese case-control population. Mol. Psychiatry 9, 981–983.

Owen, M.J., Holmans, P., McGuffin, P., 1997. Association studies in psychiatric genetics. Mol. Psychiatry 2, 270–273.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.

Schwab, S.G., Hoefgen, B., Hanses, C., Hassenbach, M.B., Albus, M., Lerer, B., et al., 2005. Further evidence for association of variants in the AKT1 gene with schizophrenia in a sample of European sib-pair families. Biol. Psychiatry 58, 446–450.

The International HapMap Consortium, 2005. A haplotype map of the human genome. Nature 437, 1299–1320.

Williams, H.J., Glaser, B., Williams, N.M., Norton, N., Zammit, S., MacGregor, S., et al., 2005a. No association between schizophrenia and polymorphisms in COMT in two large samples. Am. J. Psychiatry 162, 1736–1738.

Williams, N.M., O'Donovan, M.C., Owen, M.J., 2005b. Is the dysbindin gene (DTNBP1) a susceptibility gene for schizophrenia? Schizophr. Bull. 31, 800–805.

Wing, J.K., Cooper, J.E., Satorius, N., 1974. The Measurement and Classification of Psychiatric Illness. Cambridge University Press, Cambridge, UK.

Wing, J.K., Babor, T., Brugha, T., 1990. SCAN: schedules for the clinical assessment in neuropsychiatry. Arch. Gen. Psychiatry 47, 589–593.

Zhao, J.H., Curtis, D., Sham, P.C., 2000. Model-free analysis and permutation tests for allelic associations. Hum. Hered. 50, 133–139.

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Association study of the *G72* gene with schizophrenia in a Japanese population: A multicenter study

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NMDA receptor

ABSTRACT

G72 is one of the most widely tested genes for association with schizophrenia. As G72 activates the D-amino acid oxidase (DAO), *G72* is termed D-amino acid oxidase activator (DAOA). The aim of this study is to investigate the association between *G72* and schizophrenia in a Japanese population, using the largest sample size to date (1774 patients with schizophrenia and 2092 healthy controls). We examined eight single nucleotide polymorphisms (SNPs), which had been associated with schizophrenia in previous studies. We found nominal evidence for association of alleles, M22/rs778293, M23/rs3918342 and M24/rs1421292, and the genotype of M22/rs778293 with schizophrenia, although there was no association of allele or genotype in the other five SNPs. We also found nominal haplotypic association, including M15/rs2391191 and M19/rs778294 with schizophrenia. However, these associations were no longer positive after correction for multiple testing. We conclude that *G72* might not play a major role in the risk for schizophrenia in the Japanese population.

1. Introduction

Schizophrenia (MIM181500) is a common neuropsychiatric disorder affecting 0.5–1% of the general population worldwide. Family, twin, and adoption studies of schizophrenia have indicated that there is a strong genetic factor with an estimated heritability of approximately 80% (Cardno and Gottesman, 2000). Several genome-wide linkage scan

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studies of whole-genome linkage scans show suggestive linkages to schizophrenia on chromosomes 1q, 3p, 5q, 6p, 8p, 11q, 13q, 14p, 20q and 22q (Owen et al., 2004). Chumakov et al. (2002) focused on chromosome region 13q22–q34, which have suggested by a number of linkage studies (Blouin et al., 1998; Brzustowicz et al., 1999; Chumakov et al., 2002; Lin et al., 1995). They built a map of 191 single nucleotide polymorphisms (SNPs) in a 5-Mb segment on 13q34 and found robust evidence for genetic association between schizophrenia and several SNPs in the narrowed 65-kb region. Two overlapping genes, *G72* (MIN 607408) and *G30* (MIN 607415), which are transcribed in opposite directions and span approximately 29 and 47 kb of genomic sequences, were annotated in this region (Chumakov et al., 2002). In vitro translation of these genes resulted in a product for *G72* only. Chumakov et al. (2002) demonstrated that the *G72* protein (i.e. LG72), which is only known in higher primates, acts as an activator of the *DAO* protein. The *G72* protein was therefore referred to as *DAO* activator (*DAOA*). Gene expression analysis of *G72* in postmortem dorsolateral prefrontal cortices showed a tendency toward increased expression of *G72* mRNA in schizophrenia than that in control (Korostishevsky et al., 2004), although the reported increase of *G72* expression has yet to be replicated. Furthermore, the activity of DAO was also increased in postmortem cortices from patients with schizophrenia (Madeira et al., 2008). D-serine is an agonist at the glycine modulation site of the N-methyl-D-aspartate (NMDA) -type glutamate receptor and plays a role in neuronal migration and cell death (Scolari and Acosta, 2007). As DAO oxidizes and degrades D-serine, DAO is considered to modulate NMDA function in cortex. Lower serum level of D-serine was revealed in patients with schizophrenia as compared to that in healthy controls. Furthermore, administration of D-serine as add-on medication reduced parts of the symptoms of schizophrenia (Boks et al., 2007). Chumakov et al. (2002) hypothesized that the activation of DAO activity by a *G72* protein product might promote degradation of D-serine and cause a hypofunction of glutamate-signaling through the NMDA receptor in schizophrenia. However, the potential relationship between *G72* and NMDA receptor system still lacks supporting evidence.

Significant associations of *G72* with schizophrenia have been reported in various populations other than Japanese, such as French Canadians, Russians, German, Palestinian Arabs, South African, Ashkenazic Jewish, Chinese, Taiwanese, Scottish, Korean and Irish (Addington et al., 2004; Chumakov et al., 2002; Corvin et al., 2007; Fallin et al., 2005; Hall et al., 2004; Hong et al., 2006; Korostishevsky et al., 2004, 2006; Ma et al., 2006; Schumacher et al., 2004; Shin et al., 2007; Shinkai et al., 2007; Wang et al., 2004; Yue et al., 2006, 2007; Zou et al., 2005). The majority of replication studies of *G72* have indicated significant associations of alleles, genotypes or haplotypes with schizophrenia. However, a minority have reported no association between *G72* and schizophrenia (Bakker et al., 2007; Goldberg et al., 2006; Liu et al., 2006; Mulle et al., 2005; Sanders et al., 2008; Vilella et al., 2008; Williams et al., 2006; Wood et al., 2007). Associations of this gene were also reported with bipolar disorder (Chen et al., 2004; Hattori et al., 2003; Prata et al., 2008; Schumacher et al., 2004; Williams et al., 2006), major depression (Rietschel et al., 2008) and panic disorder (Schumacher et al., 2005).

In this study, we examined possible association between G72 polymorphisms and schizophrenia in a large Japanese population.

2. Materials and methods

2.1. Subjects

The subjects for this study consisted of 1774 patients with schizophrenia [males: 55.5%, mean age of 45.6 years (SD 15.1)] and 2092 healthy controls [males: 49.3%, mean age of 45.0 years (SD 19.7)], which is the largest sample size to date for *G72* association study. There was no significant difference in age between patients and controls groups ($P=0.30$), while the sex ratio differed significantly between groups ($P=0.00014$). All subjects were biologically unrelated Japanese and were recruited at four geographic regions, which were located on the main islands in Japan: Osaka, Aichi, Tokushima and Tokyo. There is little possibility for great ethnic/genetic difference among these regions for feature of homogeneous race in Japan (Yamaguchi-Kabata et al., 2008). Cases were recruited from both outpatients and inpatients at university hospitals and related psychiatric facilities. Controls, including hospital and institutional staffs, were recruited from local advertisements. Each patient with schizophrenia had received a diagnosis and assessment by at least two trained psychiatrists as a part of routine clinical diagnosis and treatment at the university hospitals and the related psychiatric facilities, according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on unstructured clinical interviews and other available information including medical records and other research assessments. No patient was diagnosed on the basis of medical records alone. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals who had received psychiatric medication. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University, Fujita Health University, Nagoya University, Tokushima University and Juntendo University.

2.2. SNP genotyping and genomic sequencing

Eight SNPs, rs3916965 (M12), rs3916967 (M14), rs2391191 (M15), rs778294 (M19), rs3916970 (M20), rs778293 (M22), rs3918342 (M23) and rs1421292 (M24), were selected from the genomic region of the *G72* gene and its flanking regions. The designations of the SNPs in parentheses are according to Chumakov et al. (2002). To examine the association between schizophrenia and previously associated SNPs in a Japanese cohort, we chose eight SNPs, which had been associated with schizophrenia in previous studies, although our study design using these SNPs does not provide complete *G72* gene coverage. The positions of the eight SNPs analyzed in the present study are indicated in Supplementary Fig. 1. Venous blood was collected from the subjects and genomic DNA was

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Table 1
SNP genotype and allele distribution in patients with schizophrenia and controls

Marker position		M/m[c]	SCZ (%)			CON (%)			MAF		Genotypic	Allelic	OR
SNP number[a]	Kb[b]		M/M	M/m	m/m	M/M	M/m	m/m	SCZ (%)	CON (%)	*P*-value[d] (*df*=2)	*P*-value[d] (*df*=1)	
M12	0	A/G	57.1	35.5	7.4	55.9	37.6	6.5	25.1	25.3	0.26	0.87	0.99
M14	14	G/A	55.3	36.7	8.0	53.7	39.2	7.1	26.3	26.7	0.21	0.75	0.98
M15	2	A/G	55.1	37.0	7.9	54.3	38.8	6.9	26.4	26.3	0.34	0.91	1.01
M19	23	G/A	72.9	24.4	2.7	71.5	25.9	2.6	14.9	15.5	0.55	0.43	0.95
M20	12	A/G	39.0	46.1	14.9	40.9	45.3	13.8	38.0	36.4	0.4	0.17	1.08
M22	15	A/G	54.7	38.7	6.6	58.9	35.0	6.1	26.0	23.6	**0.034**	**0.019**	1.13
M23	17	T/C	31.7	49.4	18.9	34.7	48.3	17.0	43.6	41.2	0.09	**0.030**	1.11
M24	12	A/T	24.9	50.6	24.5	28.1	49.0	23.0	49.8	47.4	0.07	**0.037**	1.10

SCZ, patients with schizophrenia; CON, healthy controls; m, minor allele; M, major allele; MAF, minor allele frequency; OR, odds ratio.
[a]The db SNP IDs equivalent to the M-SNP IDs designed by Chumakov et al. (2002) are the following: M12 (rs3916965), M14 (rs3916967), M15 (rs2391191), M19 (rs778294), M20 (rs3916970), M22 (rs778293), M23 (rs3918342), and M24 (rs1421292).
[b]Distances inter-SNPs are shown (Kb).
[c]The first shown alleles are major allele. All the alleles are represented according to the forward DNA sequence to make them comparable with the previous published data.
[d]Significant *P*-values (< 0.05) are in bold face.

extracted from whole blood according to standard procedures. Genotyping of the SNPs was carried out using TaqMan assays (Applied Biosystems, Foster City, California, USA). TaqMan probes and Universal PCR Master Mix were obtained from Applied Biosystems. A 5-μl total reaction volume was used, and allelic-specific fluorescence was measured using an ABI PRISM 7900 Sequence Detector System (Applied Biosystems). In addition, we genotyped eight SNPs in 32 randomly selected subjects (64 chromosomes) by a direct DNA sequencing method to check for typing errors by the TaqMan method. We confirmed that all genotypes determined by the direct sequencing method were in agreement with the genotypes of the TaqMan methods for all eight SNPs. Detailed information on the PCR conditions and the primer pairs are shown in Supplementary Methods and Supplementary Table 1.

2.3. Statistical analysis

Statistical analysis was performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan). The presence of Hardy–Weinberg equilibrium (HWE) was examined by using the χ^2 test for goodness of fit. The statistical significance of HWE analysis was defined at $P < 0.01$. The allelic and genotypic distributions of *G72* polymorphisms between patients and controls were analyzed using χ^2 tests for independence. We performed correction for multiple testing in single marker analysis by using the SNPSpD program (Nyholt, 2004). Pairwise linkage disequilibrium (LD) analysis, expressed by D' values, was applied to detect the intermarker relationship in each group using the Haploview software (http://www.broad.mit.edu/mpg/haploview/contact.php). Haplotype frequencies were estimated by the method of maximum likelihood from the genotyping data through the use of the Expectation-Maximization algorithm. Rare haplotypes found in less than 3% of both patients and controls were excluded from the association analysis. We performed 10,000 permutations for the most significant test to determine an empirical significance. We used a 2- to 5-window fashion analysis. Bonferroni corrections were applied for multiple comparisons of the haplotype analysis. All *P*-values reported are two tailed. Statistical significance was defined at $P < 0.05$.

2.4. Power analysis

We performed power calculations using the Power Calculator for Two Stage Association Studies (http://www.sph.umich.edu/csg/abecasis/CaTS/) (Skol et al., 2006). Power estimates were based on allele frequencies of the associated markers ranging from 0.15 (M19) to 0.38 (M20), the odds ratio ranging from 1.33 (M14) to 1.46 (M12) for the markers indicated by Chumakov et al. (2002) and an alpha level of 0.05. Power was calculated under prevalence of 0.01 using an additive or a multiplicative model, assuming various degrees of allele frequencies and the odds ratios of the markers.

3. Results

Our sample size of 1774 cases and 2092 controls had sufficient power (>0.99) to detect an effect of the odds ratio

Table 2
Haplotype analysis of G72 between patients and controls

		Haplotypic global *P* value			
		Window size			
LD[a]	SNP IDs[b]	2	3	4	5
	M12 (rs3916965)				
		0.93			
block I	M14 (rs3916967)		0.97		
		0.92		**0.03**	
	M15 (rs2391191)		**0.03**		0.05
		0.03		0.06	
	M19 (rs778294)		0.08		**0.03**
		0.04		0.06	
	M20 (rs3916970)		0.15		0.27
		0.23		0.47	
	M22 (rs778293)		0.28		0.25
		0.09		0.32	
block II	M23 (rs3918342)		0.06		
		0.10			
	M24 (rs1421292)				

LD, linkage disequilibrium.
[a]According to the result of LD analysis, we divided tightly linked SNPs into two LD blocks: block I (M12, M14 and M15), block II (M22, M23 and M24).
[b]The db SNP IDs equivalent to the M-SNP IDs designed by Chumakov et al. (2002) are shown in parentheses.
Haplotypes with frequencies <3% in each group are excluded.
Significant *P*-values (<0.05) are in bold face.

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(1.33 or more) described in the initial report for each SNP (Chumakov et al., 2002). Genotype and allele frequencies of eight SNPs located in the *G72* gene and the flanking regions are shown in Table 1. Genotyping completeness ranged from 98.5% (M15) to 99.5% (M12). No deviation from HWE was detected in cases and controls (data not shown). Significant differences in the genotype frequency of M22 (χ^2=6.75, P=0.034) and in the allele frequencies of M22 (χ^2=5.48, P=0.019), M23 (χ^2=4.72, P=0.030) and M24 (χ^2=4.35, P=0.037) between patients and controls were observed. However, the associations did not survive after correction for multiple testing (the effective number of independent marker loci: 6.0, M22 allelic association: P=0.11 after SNPSpD correction). There was no allelic or genotypic association of the other five SNPs with schizophrenia.

Haplotype analysis showed associations in two-marker haplotypes: M15-M19 (global P=0.03) and M19–M20 (global P=0.04), three-marker haplotype: M14–M15–M19 (global P=0.03), four-marker haplotype: M12–M14–M15–M19 (global P=0.03), and five-marker haplotype: M14–M15–M19–M20–M22 (global P=0.03) (Table 2). This weak evidence for association became negative after correction for multiple testing (22 independent global test, M15–M19 haplotypic association: P=0.66 after Bonferroni correction).

M22 showed a strong LD with M24 and a moderate LD with M23 in controls, and similar LD results were obtained in patients (Supplementary Fig. 1). The LD pattern of our data was similar to that of other ethnic groups in the previous studies. The two strong LD structures (Block 1 and Block 2) observed in the present study were similar to those observed in previous studies (Detera-Wadleigh and McMahon, 2006; Li and He, 2007; Ma et al., 2006).

4. Discussion

The purpose of the present study was to investigate the association between *G72* polymorphisms and schizophrenia in a large Japanese population, comparable to the sample size included in the meta-analysis of Li and He (2007). Eight *G72* SNPs, which have been associated with schizophrenia in previous studies, were examined in case-control subjects (Detera-Wadleigh and McMahon, 2006; Li and He, 2007; Shi et al., 2008). We failed to replicate the association of any *G72* polymorphism (M12, M14, M15, M19, M20, M22, M23 and M24) with schizophrenia after correction for multiple testing. Power analysis showed that our subjects had sufficient power (>0.99) to detect an effect of the odds ratio (1.33 or more) for each SNP shown in the original study. The findings of the power calculation did not support the hypothesis that the eight SNPs in the *G72* gene are associated with schizophrenia in Japanese population.

Nominal associations of the alleles, M22, M23 and M24, and the genotype of M22 with schizophrenia in this study were no longer positive after correction for multiple testing. However, we discuss the direction of the association in the SNPs, as there are considerable discrepancies among studies. These three SNPs are located from 25.8 Kb to 54.8 Kb 3' downstream of the last exon (exon 5) of *G72*. These SNPs form a highly strong LD block whose pattern is similar among different ethnic groups. The first study by Chumakov et al. reported that the minor allele G of M22 was less frequent in Canadian patients with schizophrenia (31%) than in controls (40%). However, the following studies have indicated the reverse direction of the association of M22 compared with the original study (patients vs. controls: Ma et al.; 47.5 vs. 39.1 in Scottish samples, 41.1 vs. 34.6 in Chinese samples). Our results indicated that the minor allele G of M22 was enriched in patients with schizophrenia (26.0) than in control (23.6). Consistent to the discrepancies among studies (different direction in European populations and same direction in Asian population), recent meta-analysis has reported significant evidence for the association of M22 with schizophrenia in Asian population, but not in European population (Ma et al., 2006; Shi et al., 2008). The direction of association of M23 has shown significant heterogeneity between individual studies in European populations (C allele at M23, patients vs. controls: Chumakov et al.,2002; 43 vs. 51 in Canadian samples, 40 vs. 49 in Russian sample, Korostishevsky et al., 2006; 37.7 vs. 56.7 in Ashkenazi Jewish samples, Schumacher et al., 2004; 53 vs. 46 in German samples, Ma et al., 2006; 59.3 vs. 46.4 in Scottish samples) (Shi et al., 2008). Our results (patients vs. controls: 43.6 vs. 41.2) were consistent with the direction of the association of M23 in an Asian population (Shi et al., 2008; 53.3 vs. 52.4), although the statistical significance was not evident. The direction of association of M24 among the previous studies was identical (patients vs. controls: Chumakov et al., 2002; 55 vs. 47 in Canadian samples, Schumacher et al., 2004; 56 vs. 50 in German samples). We firstly examined a possible association of M24 with schizophrenia in an Asian population and did not find association with schizophrenia (the frequency of the T allele at M24 in patients: 49.8 and controls: 47.4). This suggests that M24 is not likely to have ethnic heterogeneity between Asian and European populations.

Recently, a large scale genome-wide association study (GWAS) using 479 cases and 2937 controls reported that 12 SNPs (odd ratios ranging 1.27–2.06, $P < 1 \times 10^{-5}$) were associated with schizophrenia in the first analysis (O'Donovan et al., 2008). The subsequent replication studies using 16,726 of total subjects showed the evidence for association with three of the 12 SNPs ($P < 1 \times 10^{-5}$) (O'Donovan et al., 2008). They reported the odd ratios 1.12–1.16 in the three positive SNPs in the overall analysis (O'Donovan et al., 2008). The sample size in the first analysis of the GWAS had power (>0.85) to detect an effect of the odd ratio (1.33 or more) for each SNP in the original *G72* study. However, association between SNPs in *G72* and schizophrenia was not detected in the study. Failure to detect association signal does not provide conclusive exclusion of any given gene identified so far. The discrepancies among previous studies and the present study might be explained by differences in ethnic heterogeneity, phenotypic heterogeneity or study designs, such as sample size and case-control versus family-based association study. Factors like incomplete coverage of common variants, inadequate power, allelic and locus heterogeneity could all affect our ability to detect genetic association.

In conclusion, the present study did not support a strong association of the *G72* gene with schizophrenia in a Japanese population. Three SNPs and several haplotypes gave nominal evidence for association and this did not survive correction for multiple testing. *G72* is not likely to be a major susceptibility gene for schizophrenia in this Japanese population.

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Contributors

R. Hashimoto supervised the entire project, collected the data, wrote the manuscript, was critically involved in the design, analysis and interpretation of the data and was responsible for performing the literature review. K. Ohi was critically involved in the collection and analysis of the data, and contributed to the editing of the final manuscript and contributed intellectually to the interpretation of the data. Y. Yasuda, T. Yoshida, H. Takahashi, N. Iike, M. Fukumoto, H. Takamura, M. Iwase, K. Kamino, R. Ishii, H. Kazui, R. Sekiyama, Y. Kitamura, M. Azechi, K. Ikezawa, R. Kurimoto, E. Kamagata, H. Tanimukai, S. Tagami, T. Morihara, M. Ogasawara, M. Okochi, H. Tokunaga, S. Numata, M. Ikeda, T. Ohnuma, T. Fukunaga, T. Tanaka, T. Kudo, S. Ueno, H. Arai, T. Ohmori, N. Iwata, N. Ozaki and M. Takeda were heavily involved in the collection of the majority of the data and contributed intellectually to the interpretation of the data. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2009.01.019.

References

Addington, A.M., Gornick, M., Sporn, A.L., Gogtay, N., Greenstein, D., Lenane, M., Gochman, P., Baker, N., Balkissoon, R., Vakkalanka, R.K., Weinberger, D.R., Straub, R.E., Rapoport, J.L., 2004. Polymorphisms in the 13q33.2 gene G72/G30 are associated with childhood-onset schizophrenia and psychosis not otherwise specified. Biol. Psychiatry 55, 976–980.

Bakker, S.C., Hoogendoorn, M.L., Hendriks, J., Verzijlbergen, K., Caron, S., Verduijn, W., Selten, J.P., Pearson, P.L., Kahn, R.S., Sinke, R.J., 2007. The *PIP5K2A* and *RGS4* genes are differentially associated with deficit and non-deficit schizophrenia. Genes Brain Behav. 6, 113–119.

Blouin, J.L., Dombroski, B.A., Nath, S.K., Lasseter, V.K., Wolyniec, P.S., Nestadt, G., Thornquist, M., Ullrich, G., McGrath, J., Kasch, L., Lamacz, M., Thomas, M.G., Gehrig, C., Radhakrishna, U., Snyder, S.E., Balk, K.G., Neufeld, K., Swartz, K.L., DeMarchi, N., Papadimitriou, G.N., Dikeos, D.G., Stefanis, C.N., Chakravarti, A., Childs, B., Housman, D.E., Kazazian, H.H., Antonarakis, S., Pulver, A.E., 1998. Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. Nat. Genet. 20, 70–73.

Boks, M.P., Rietkerk, T., van de Beek, M.H., Sommer, I.E., de Koning, T.J., Kahn, R.S., 2007. Reviewing the role of the genes G72 and DAAO in glutamate neurotransmission in schizophrenia. Eur. Neuropsychopharmacol. 17, 567–572.

Brzustowicz, L.M., Honer, W.G., Chow, E.W., Little, D., Hogan, J., Hodgkinson, K., Bassett, A.S., 1999. Linkage of familial schizophrenia to chromosome 13q32. Am. J. Hum. Genet. 65, 1096–1103.

Cardno, A.G., Gottesman, 2000. Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am. J. Med. Genet. 97, 12–17.

Chen, Y.S., Akula, N., Detera-Wadleigh, S.D., Schulze, T.G., Thomas, J., Potash, J.B., DePaulo, J.R., McInnis, M.G., Cox, N.J., McMahon, F.J., 2004. Findings in an independent sample support an association between bipolar affective disorder and the G72/G30 locus on chromosome 13q33. Mol. Psychiatry 9, 87–92.

Chumakov, I., Blumenfeld, M., Guerassimenko, O., Cavarec, L., Palicio, M., Abderrahim, H., Bougueleret, L., Barry, C., Tanaka, H., La Rosa, P., Puech, A., Tahri, N., Cohen-Akenine, A., Delabrosse, S., Lissarrague, S., Picard, F.P., Maurice, K., Essioux, L., Millasseau, P., Grel, P., Debailleul, V., Simon, A.M., Caterina, D., Dufaure, I., Malekzadeh, K., Belova, M., Luan, J.J., Bouillot, M., Sambucy, J.L., Primas, G., Saumier, M., Boubkiri, N., Martin-Saumier, S., Nasroune, M., Peixoto, H., Delaye, A., Pinchot, V., Bastucci, M., Guillou, S., Chevillon, M., Sainz-Fuertes, R., Meguenni, S., Aurich-Costa, J., Cherif, D., Gimalac, A., Van Duijn, C., Gauvreau, D., Ouellette, G., Fortier, I., Raelson, J., Sherbatich, T., Riazanskaia, N., Rogaev, E., Raeymaekers, P., Aerssens, J., Konings, F., Luyten, W., Macciardi, F., Sham, P.C., Straub, R.E., Weinberger, D.R., Cohen, N., Cohen, D., 2002. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 99, 13675–13680.

Corvin, A., McGhee, K.A., Murphy, K., Donohoe, G., Nangle, J.M., Schwaiger, S., Kenny, N., Clarke, S., Meagher, D., Quinn, J., Scully, P., Baldwin, P., Browne, D., Walsh, C., Waddington, J.L., Morris, D.W., Gill, M., 2007. Evidence for association and epistasis at the DAOA/G30 and D-amino acid oxidase loci in an Irish schizophrenia sample. Am. J. Med. Genet. B Neuropsychiatr. Genet. 144, 949–953.

Detera-Wadleigh, S.D., McMahon, F.J., 2006. G72/G30 in schizophrenia and bipolar disorder: review and meta-analysis. Biol. Psychiatry 60, 106–114.

Fallin, M.D., Lasseter, V.K., Avramopoulos, D., Nicodemus, K.K., Wolyniec, P.S., McGrath, J.A., Steel, G., Nestadt, G., Liang, K.Y., Huganir, R.L., Valle, D., Pulver, A.E., 2005. Bipolar I disorder and schizophrenia: a 440-single-nucleotide polymorphism screen of 64 candidate genes among Ashkenazi Jewish case-parent trios. Am. J. Hum. Genet. 77, 918–936.

Goldberg, T.E., Straub, R.E., Callicott, J.H., Hariri, A., Mattay, V.S., Bigelow, L., Coppola, R., Egan, M.F., Weinberger, D.R., 2006. The G72/G30 gene complex and cognitive abnormalities in schizophrenia. Neuropsychopharmacology 31, 2022–2032.

Hall, D., Gogos, J.A., Karayiorgou, M., 2004. The contribution of three strong candidate schizophrenia susceptibility genes in demographically distinct populations. Genes Brain Behav. 3, 240–248.

Hattori, E., Liu, C., Badner, J.A., Bonner, T.I., Christian, S.L., Maheshwari, M., Detera-Wadleigh, S.D., Gibbs, R.A., Gershon, E.S., 2003. Polymorphisms at the G72/G30 gene locus, on 13q33, are associated with bipolar disorder in two independent pedigree series. Am. J. Hum. Genet. 72, 1131–1140.

Hong, C.J., Hou, S.J., Yen, F.C., Liou, Y.J., Tsai, S.J., 2006. Family-based association study between G72/G30 genetic polymorphism and schizophrenia. Neuroreport 17, 1067–1069.

Korostishevsky, M., Kaganovich, M., Cholostoy, A., Ashkenazi, M., Ratner, Y., Dahary, D., Bernstein, J., Bening-Abu-Shach, U., Ben-Asher, E., Lancet, D., Ritsner, M., Navon, R., 2004. Is the G72/G30 locus associated with schizophrenia? Single nucleotide polymorphisms, haplotypes, and gene expression analysis. Biol. Psychiatry 56, 169–176.

Korostishevsky, M., Kremer, I., Kaganovich, M., Cholostoy, A., Murad, I., Muhaheed, M., Bannoura, I., Rietschel, M., Dobrusin, M., Bening-Abu-Shach, U., Belmaker, R.H., Maier, W., Ebstein, R.P., Navon, R., 2006. Transmission disequilibrium and haplotype analyses of the G72/G30 locus: suggestive linkage to schizophrenia in Palestinian Arabs living in the North of Israel. Am. J. Med. Genet. B Neuropsychiatr. Genet. 141, 91–95.

Li, D., He, L., 2007. G72/G30 genes and schizophrenia: a systematic meta-analysis of association studies. Genetics 175, 917–922.

Lin, M.W., Curtis, D., Williams, N., Arranz, M., Nanko, S., Collier, D., McGuffin, P., Murray, R., Owen, M., Gill, M., Powell, J., 1995. Suggestive evidence for linkage of schizophrenia to markers on chromosome 13q14.1–q32. Psychiatr. Genet. 5, 117–126.

Liu, Y.L., Fann, C.S., Liu, C.M., Chang, C.C., Wu, J.Y., Hung, S.I., Liu, S.K., Hsieh, M.H., Hwang, T.J., Chan, H.Y., Chen, J.J., Faraone, S.V., Tsuang, M.T., Chen, W.J., Hwu, H.G., 2006. No association of G72 and D-amino acid oxidase genes with schizophrenia. Schizophr. Res. 87, 15–20.

Ma, J., Qin, W., Wang, X.Y., Guo, T.W., Bian, L., Duan, S.W., Li, X.W., Zou, F.G., Fang, Y.R., Fang, J.X., Feng, G.Y., Gu, N.F., St Clair, D., He, L., 2006. Further evidence for the association between G72/G30 genes and schizophrenia in two ethnically distinct populations. Mol. Psychiatry 11, 479–487.

Madeira, C., Freitas, M.E., Vargas-Lopes, C., Wolosker, H., Panizzutti, R., 2008. Increased brain D-amino acid oxidase (DAAO) activity in schizophrenia. Schizophr. Res. 101, 76–83.

Mulle, J.G., Chowdari, K.V., Nimgaonkar, V., Chakravarti, A., 2005. No evidence for association to the G72/G30 locus in an independent sample of schizophrenia families. Mol. Psychiatry 10, 431–433.

Nyholt, D.R., 2004. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am. J. Hum. Genet. 74, 765–769.

O'Donovan, M.C., Craddock, N., Norton, N., Williams, H., Peirce, T., Moskvina, V., Nikolov, I., Hamshere, M., Carroll, L., Georgieva, L., Dwyer, S., Holmans, P., Marchini, J.L., Spencer, C.C., Howie, B., Leung, H.T., Hartmann, A.M., Moller, H.J., Morris, D.W., Shi, Y., Feng, G., Hoffmann, P., Propping, P., Vasilescu, C., Maier, W., Rietschel, M., Zammit, S., Schumacher, J., Quinn, E.M., Schulze, T. G., Williams, N.M., Giegling, I., Iwata, N., Ikeda, M., Darvasi, A., Shifman, S., He, L., Duan, J., Sanders, A.R., Levinson, D.F., Gejman, P.V., Buccola, N.G.,

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Mowry, B.J., Freedman, R., Amin, F., Black, D.W., Silverman, J.M., Byerley, W.F., Cloninger, C.R., Cichon, S., Nothen, M.M., Gill, M., Corvin, A., Rujescu, D., Kirov, G., Owen, M.J., 2008. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat. Genet. 40, 1053–1055.

Owen, M.J., Williams, N.M., O'Donovan, M.C., 2004. The molecular genetics of schizophrenia: new findings promise new insights. Mol. Psychiatry 9, 14–27.

Prata, D., Breen, G., Osborne, S., Munro, J., St Clair, D., Collier, D., 2008. Association of DAO and *G72*(DAOA)/*G30* genes with bipolar affective disorder. Am. J. Med. Genet. B Neuropsychiatr. Genet. 147B, 914–917.

Rietschel, M., Beckmann, L., Strohmaier, J., Georgi, A., Karpushova, A., Schirmbeck, F., Boesshenz, K.V., Schmal, C., Burger, C., Jamra, R.A., Schumacher, J., Hofels, S., Kumsta, R., Entringer, S., Krug, A., Markov, V., Maier, W., Propping, P., Wust, S., Kircher, T., Nothen, M.M., Cichon, S., Schulze, T.G., 2008. G72 and its association with major depression and neuroticism in large population-based groups from Germany. Am. J. Psychiatry 165, 753–762.

Sanders, A.R., Duan, J., Levinson, D.F., Shi, J., He, D., Hou, C., Burrell, G.J., Rice, J.P., Nertney, D.A., Olincy, A., Rozic, P., Vinogradov, S., Buccola, N.G., Mowry, B.J., Freedman, R., Amin, F., Black, D.W., Silverman, J.M., Byerley, W.F., Crowe, R.R., Cloninger, C.R., Martinez, M., Gejman, P.V., 2008. No significant association of 14 candidate genes with schizophrenia in a large European ancestry sample: implications for psychiatric genetics. Am. J. Psychiatry 165, 497–506.

Schumacher, J., Jamra, R.A., Freudenberg, J., Becker, T., Ohlraun, S., Otte, A.C., Tullius, M., Kovalenko, S., Bogaert, A.V., Maier, W., Rietschel, M., Propping, P., Nothen, M.M., Cichon, S., 2004. Examination of G72 and D-amino-acid oxidase as genetic risk factors for schizophrenia and bipolar affective disorder. Mol. Psychiatry 9, 203–207.

Schumacher, J., Abou Jamra, R., Becker, T., Klopp, N., Franke, P., Jacob, C., Sand, P., Fritze, J., Ohlraun, S., Schulze, T.G., Rietschel, M., Illig, T., Propping, P., Cichon, S., Deckert, J., Nothen, M.M., 2005. Investigation of the DAOA/G30 locus in panic disorder. Mol. Psychiatry 10, 428–429.

Scolari, M.J., Acosta, G.B., 2007. D-serine: a new word in the glutamatergic neuro-glial language. Amino Acids 33, 563–574.

Shi, J., Badner, J.A., Gershon, E.S., Liu, C., 2008. Allelic association of G72/G30 with schizophrenia and bipolar disorder: a comprehensive meta-analysis. Schizophr. Res. 98, 89–97.

Shin, H.D., Park, B.L., Kim, E.M., Lee, S.O., Cheong, H.S., Lee, C.H., Kim, S.G., Sohn, J.W., Park, C.S., Kim, J.W., Kim, B.H., Kim, I.Y., Choi, I.G., Woo, S.I., 2007. Association analysis of G72/G30 polymorphisms with schizophrenia in the Korean population. Schizophr. Res. 96, 119–124.

Shinkai, T., De Luca, V., Hwang, R., Muller, D.J., Lanktree, M., Zai, G., Shaikh, S., Wong, G., Sicard, T., Potapova, N., Trakalo, J., King, N., Matsumoto, C., Hori, H., Wong, A.H., Ohmori, O., Macciardi, F., Nakamura, J., Kennedy, J.L., 2007. Association analyses of the DAOA/G30 and D-amino-acid oxidase genes in schizophrenia: further evidence for a role in schizophrenia. Neuromolecular Med. 9, 169–177.

Skol, A.D., Scott, L.J., Abecasis, G.R., Boehnke, M., 2006. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat. Genet. 38, 209–213.

Vilella, E., Costas, J., Sanjuan, J., Guitart, M., De Diego, Y., Carracedo, A., Martorell, L., Valero, J., Labad, A., De Frutos, R., Najera, C., Molto, M.D., Toirac, I., Guillamat, R., Brunet, A., Valles, V., Perez, L., Leon, M., de Fonseca, F.R., Phillips, C., Torres, M., 2008. Association of schizophrenia with DTNBP1 but not with DAO, DAOA, NRG1 and RGS4 nor their genetic interaction. J. Psychiatr. Res. 42, 278–288.

Wang, X., He, G., Gu, N., Yang, J., Tang, J., Chen, Q., Liu, X., Shen, Y., Qian, X., Lin, W., Duan, Y., Feng, G., He, L., 2004. Association of G72/G30 with schizophrenia in the Chinese population. Biochem. Biophys. Res. Commun. 319, 1281–1286.

Williams, N.M., Green, E.K., Macgregor, S., Dwyer, S., Norton, N., Williams, H., Raybould, R., Grozeva, D., Hamshere, M., Zammit, S., Jones, L., Cardno, A., Kirov, G., Jones, I., O'Donovan, M.C., Owen, M.J., Craddock, N., 2006. Variation at the DAOA/G30 locus influences susceptibility to major mood episodes but not psychosis in schizophrenia and bipolar disorder. Arch. Gen. Psychiatry 63, 366–373.

Wood, L.S., Pickering, E.H., Dechairo, B.M., 2007. Significant support for DAO as a schizophrenia susceptibility locus: examination of five genes putatively associated with schizophrenia. Biol. Psychiatry 61, 1195–1199.

Yamaguchi-Kabata, Y., Nakazono, K., Takahashi, A., Saito, S., Hosono, N., Kubo, M., Nakamura, Y., Kamatani, N., 2008. Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: effects on population-based association studies. Am. J. Hum. Genet. 83, 445–456.

Yue, W., Liu, Z., Kang, G., Yan, J., Tang, F., Ruan, Y., Zhang, J., Zhang, D., 2006. Association of G72/G30 polymorphisms with early-onset and male schizophrenia. Neuroreport 17, 1899–1902.

Yue, W., Kang, G., Zhang, Y., Qu, M., Tang, F., Han, Y., Ruan, Y., Lu, T., Zhang, J., Zhang, D., 2007. Association of DAOA polymorphisms with schizophrenia and clinical symptoms or therapeutic effects. Neurosci. Lett. 416, 96–100.

Zou, F., Li, C., Duan, S., Zheng, Y., Gu, N., Feng, G., Xing, Y., Shi, J., He, L., 2005. A family-based study of the association between the G72/G30 genes and schizophrenia in the Chinese population. Schizophr. Res. 73, 257–261.

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Impaired regional hemodynamic response in schizophrenia during multiple prefrontal activation tasks: A two-channel near-infrared spectroscopy study

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ABSTRACT

In schizophrenia, dysfunction of the prefrontal cortex (PFC), regarded as a core feature of the disease, has been investigated by different neuroimaging methods. Near infrared spectroscopy (NIRS), a novel neurophysiological method, is being increasingly used in the investigation of frontal dysfunction in schizophrenia. However, NIRS measurements during multiple frontal activation tasks have been rarely reported. The purpose of this study was to compare hemodynamic changes in the PFC between patients with schizophrenia and healthy controls during four different types of frontal lobe tasks using a 2-channel NIRS system. Thirty patients with schizophrenia and thirty age- and gender-matched healthy controls were enrolled in this study. In both groups, changes in oxygenated hemoglobin concentration (Δ[oxyHb]) at the bilateral forehead were measured during Verbal fluency test letter version (VFT-letter), VFT category version, Tower of Hanoi (TOH), the Sternberg and Stroop tasks. Regarding Δ[oxyHb] in PFC, a diagnosis group effect was found for VFT-letter and TOH. Significant negative correlation was found between left Δ[oxyHb] during TOH and negative and cognitive symptom scores in schizophrenia patients. Right Δ[oxyHb] during TOH also showed significant negative correlation with cognitive symptoms scores. No significant correlation between Δ[oxyHb] and clinical characteristics were observed during VFT-letter. These findings suggest that among a battery of frontal lobe tasks administered to schizophrenia patients, VFT-letter and TOH are more sensitive to detect PFC activation, as indicated by Δ[oxyHb] using a 2-channel NIRS. Taken together, these findings and those of previous neuroimaging studies suggest that VFT-letter and TOH might represent possible candidate physiological markers of prefrontal dysfunction in schizophrenia, though extensive testing in clinical settings will be necessary.

1. Introduction

Schizophrenia is a mental disorder emerging in adolescence that is typically characterized by hallucinations and delusions as well as emotional and social dysfunction. Recently, marked cognitive impairments, predominantly in memory, attention, and executive functions have been described in patients with

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