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ORIGINAL INVESTIGATION

The *AKT1* gene is associated with attention and brain morphology in schizophrenia

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Abstract

Objectives. A meta-analysis of the associations between genetic variants in the *AKT1* gene and schizophrenia found that a single nucleotide polymorphism (SNP5; rs2494732) was associated with schizophrenia in Asian populations. *Methods.* In this study, we investigated the effects of this SNP on memory and attentional performance and brain structure using magnetic resonance imaging in a Japanese population (117 patients with schizophrenia and 189 healthy subjects). *Results.* The memory performance, particularly attention/concentration score, measured by the Wechsler Memory Scale-Revised in A carriers of SNP5, which was found to be enriched in patients with schizophrenia, was lower than that in individuals with the G/G genotype. We confirmed the association of the SNP with attentional performance using the Continuous Performance Test, which assessed sustained attention and vigilance of attentional function. Patients with A allele demonstrated lower attentional performance than patients with the G/G genotype. Patients with the A allele had smaller gray matter volumes in the right inferior parietal lobule related to attentional processes and in the frontostriatal region related to different SNPs in *AKT1* than patients with the G/G genotype. *Conclusions.* Our results suggest that a genetic variant of *AKT1* might be associated with attentional deficits and brain morphological vulnerability in patients with schizophrenia.

Key words: AKT1, *schizophrenia, single nucleotide polymorphism (SNP), attention, VBM*

Introduction

V-akt murine thymoma viral oncogene homolog 1 (AKT1, also known as PKB; protein kinase B) belongs to a serine/threonine kinase family and is highly expressed in the brain. AKT1 serves as a central node in cell signaling downstream of growth factors, cytokines, and other external stimuli. AKT1 contributes to several cellular functions such as cell growth, survival, and metabolism (Grimes and Jope 2001). Several studies in rodents demonstrated involvement of AKT1 in memory formation and synaptic plasticity (Lin et al. 2001; Mizuno et al. 2003; Horwood et al. 2006; Sui et al. 2008). In addition, *Akt1*-knockout mice showed poorer working memory performance

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under dopaminergic agonist challenge (Lai et al. 2006).

Schizophrenia is a common and complex psychiatric disease with strong genetic components. Schizophrenia has an estimated heritability of approximately 80% (Cardno and Gottesman 2000; Tsuang 2000) and many genes have been implicated in the pathogenesis of schizophrenia (Sun et al. 2008). Attention and memory are specifically impaired in schizophrenia (Green 2006). The attention and memory deficits are prominent trait markers for schizophrenia, with impairments also observed in first-degree relatives (Snitz et al. 2006). Susceptibility genes have been hypothesized to contribute to the disorder at least partly by influencing cognitive performance (Weinberger et al. 2001a). The *AKT1* gene located on chromosome 14q32.32 has been reported as a susceptibility gene for schizophrenia in various populations from the United States, the UK, Ireland, Switzerland, France, Bulgaria, Australia, Iran, China and Japan (Emamian et al. 2004; Ikeda et al. 2004; Schwab et al. 2005; Bajestan et al. 2006; Norton et al. 2007; Xu et al. 2007; Shi et al. 2008; Thiselton et al. 2008; Betcheva et al. 2009; Karege et al. 2010; Mathur et al. 2010), but not in all studies (Ohtsuki et al. 2004; Ide et al. 2006; Liu et al. 2006; Turunen et al. 2007; Sanders et al. 2008; Lee et al. 2010). A genetic variant, rs2494732 (SNP5), in the *AKT1* gene was associated with schizophrenia in Asian populations in a recent meta-analysis (Shi et al. 2008). This polymorphism was also found to predict treatment response to risperidone in Japanese patients with schizophrenia (Ikeda et al. 2008). The other genetic variant, rs1130233 (SNP4), in the *AKT1* gene was associated with AKT1 protein levels in lymphoblast (Harris et al. 2005; Tan et al. 2008b). The haplotypes comprising rs1130214 (SNP2), rs3730358 (SNP3) and SNP4 was associated with AKT1 protein levels in postmortem brain tissues (Karege et al. 2010). The interactions between SNP4 and rs1076560 in the dopamine D2 or a functional polymorphism (Val158Met) in catechol-*O*-methyltransferase (*COMT*) have been also investigated (Tan et al. 2008b). The interactions between SNP4 and rs1076560 were associated with AKT1 protein levels, phosphorylation of GSK3β, cingulate response, behavioural accuracy during attentional processing and response to olanzapine treatment (Blasi et al. 2011). The interactions between SNP4 and Val158Met were related to AKT1 phosphorylation (Sei et al. 2010), prefrontal physiology during executive function, and frontostriatal gray matter volume (Tan et al. 2008b). In this study, we focused on the single marker SNP5 associated with schizophrenia in Asian populations, but not these interactions to elucidate the genotype effect as simple as possible.

Many attempts have been made to minimize clinical and genetic heterogeneity for schizophrenia. A strategy for gene discovery proposes using quantitative neurobiological traits as intermediate phenotypes instead of the diagnosis of schizophrenia (Meyer-Lindenberg and Weinberger 2006; Tan et al. 2008a). This strategy has the potential to reduce clinical and genetic heterogeneity by applying intermediate phenotypes that reflect underlying genetic vulnerability better than diagnostic categorization. Memory, attention and brain structure are potential intermediate phenotypes that bridges the gap between genotype and diagnostic categorization (Chen and Faraone 2000; Weinberger et al. 2001b; Skelley et al. 2008). In recent years, a number of attempts to explain the link between intermediate phenotypes and a specific gene have been made. Indeed, *AKT1* was shown to be associated with verbal learning and memory in twin pairs of healthy controls, in patients with schizophrenia and in patients with bipolar disorder (Pietilainen et al. 2009). *AKT1* has also been associated with behavioral accuracy during attentional processing in healthy subjects (Blasi et al. 2011) and associated with IQ/processing speed in subjects with European ancestry (Tan et al. 2008b), although one study did not find an association between *AKT1* and verbal memory, working memory, visual memory or vigilance in patients with schizophrenia (Pinheiro et al. 2007). It was also reported that *AKT1* was associated with prefrontal structures in healthy controls (Tan et al. 2008a,b). In this study, we examined possible impacts of a genetic variant (SNP5) of *AKT1* on performances of memory and attention and brain structure in a Japanese population.

Methods and materials

Subjects

The subjects for this study consisted of 117 unrelated patients with schizophrenia (50.4% males (59/58), mean age ± SD; 35.9 ± 11.5 years) and 189 unrelated healthy controls (49.2% males (93/96), mean age ± SD; 38.3 ± 12.1 years). The Wechsler Memory Scale-Revised (WMR-R) was administered to 94 patients and 121 controls (Table I). The Continuous Performance Test – Identical pairs version (CPT-IP) was administered to 60 patients and 121 controls (Table II). Neuroimaging analysis was performed for 55 patients and 159 controls (Table III). Although we attempted to examine WMS-R, CPT-IP and magnetic resonance (MR) imaging from all subjects as much as we could, all tests were available only for 26 patients with schizophrenia and 91

Table I. Demographic information for subjects included in WMS-R analysis.

Variables	Schizophrenia ($N=94$) G/G ($N=46$)	Schizophrenia ($N=94$) A carriers ($N=48$)	Schizophrenia ($N=94$) P values (z)	Control ($N=121$) G/G ($N=57$)	Control ($N=121$) A carriers ($N=64$)	Control ($N=121$) P values (z)	Group difference P values (z)
Age (years)	39.9 ± 12.0	39.2 ± 12.0	0.90 (–0.12)	35.1 ± 12.3	34.9 ± 11.5	0.98 (–0.03)	**0.0022 (–3.06)**
Sex (male/female)	26/20	23/25	0.40 (0.70)[a]	32/25	31/33	0.31 (1.04)[a]	0.99 (<0.01)[a]
Education (years)	13.8 ± 2.4	14.2 ± 2.2	0.28 (–1.07)	15.4 ± 2.3	15.6 ± 2.1	0.70 (–0.38)	**3.37×10^{-6} (–4.65)**
Estimated premorbid IQ	100.9 ± 9.9	99.9 ± 9.9	0.69 (–0.40)	106.0 ± 8.0	104.2 ± 8.5	0.30 (–1.05)	**4.77×10^{-4} (–3.49)**
CPZeq (mg/day)	601.4 ± 479.7	583.8 ± 450.7	0.97 (–0.04)	–	–	–	–
Age at onset (years)	23.5 ± 9.0	24.0 ± 7.3	0.59 (–0.55)	–	–	–	–
Duration of illness (years)	16.4 ± 10.8	15.3 ± 11.6	0.59 (–0.53)	–	–	–	–
PANSS positive symptoms	16.7 ± 5.5	17.7 ± 6.8	0.66 (–0.44)	–	–	–	–
PANSS negative symptoms	17.9 ± 7.2	18.1 ± 7.2	0.93 (–0.08)	–	–	–	–
PANSS general psychopathology	34.1 ± 9.5	36.1 ± 10.7	0.41 (–0.82)	–	–	–	–

Means ± SD and P values are shown. [a]χ^2-test. PANSS, Positive and Negative Syndrome Scale; CPZ-eq, chlorpromazine equivalent of total antipsychotics. There was no significant difference between genotypes for any variable in each genotype group. Complete demographic information was not obtained for all patients with schizophrenia (estimated premorbid IQ: A carriers, $N=43$; PANSS: A carriers, $N=46$).

Table II. Demographic information for subjects included in CPT analysis.

Variables	Schizophrenia ($N=60$) G/G ($N=35$)	Schizophrenia ($N=60$) A carriers ($N=25$)	Schizophrenia ($N=60$) P values (z)	Control ($N=121$) G/G ($N=57$)	Control ($N=121$) A carriers ($N=64$)	Control ($N=121$) P values (z)	Group difference P values (z)
Age (years)	40.0 ± 12.4	40.4 ± 11.6	0.65 (–0.46)	35.1 ± 12.3	34.9 ± 11.5	0.51 (–0.65)	**0.0035 (–2.92)**
Sex (male/female)	22/13	12/13	0.25 (1.31)[a]	32/25	31/33	0.40 (0.72)[a]	0.56 (0.34)[a]
Education (years)	14.0 ± 2.5	14.2 ± 2.0	0.47 (–0.72)	15.4 ± 2.3	15.6 ± 2.1	0.87 (–0.17)	**9.91×10^{-5} (–3.89)**
Estimated premorbid IQ	100.0 ± 10.4	99.8 ± 11.1	0.99 (–0.01)	106.0 ± 8.0	104.2 ± 8.5	0.34 (–0.95)	**0.0011 (–3.25)**
CPZeq (mg/day)	542.9 ± 477.6	544.0 ± 394.2	0.76 (–0.31)	–	–	–	–
Age at onset (years)	23.7 ± 9.2	23.9 ± 6.6	0.50 (–0.67)	–	–	–	–
Duration of illness (years)	16.3 ± 11.0	16.5 ± 12.1	0.98 (–0.02)	–	–	–	–
PANSS positive symptoms	16.8 ± 5.9	18.8 ± 7.6	0.42 (–0.80)	–	–	–	–
PANSS negative symptoms	18.5 ± 7.3	18.5 ± 8.1	0.91 (–0.11)	–	–	–	–
PANSS general psychopathology	35.5 ± 9.8	36.2 ± 11.5	0.90 (–0.13)	–	–	–	–

Means ± SD and P values are shown. [a]χ^2-test. PANSS, Positive and Negative Syndrome Scale; CPZ-eq, chlorpromazine equivalent of total antipsychotics. There was no significant difference between genotypes for any variable in each genotype group.

Table III. Demographic information for patients with schizophrenia and healthy controls included in the ROI analysis.

Variables	Schizophrenia ($N = 55$)			Control ($N = 159$)			Group difference
	G/G ($N = 25$)	A carriers ($N = 30$)	*P* values (*z*)	G/G ($N = 71$)	A carriers ($N = 88$)	*P* values (*z*)	*P* values (*z*)
Age (years)	35.3 ± 12.2	36.5 ± 11.0	0.59 (–0.53)	36.8 ± 10.9	36.7 ± 11.5	0.89 (–0.14)	0.64 (–0.47)
Sex (male/female)	15/10	14/16	0.32 (0.97)[a]	33/38	42/46	0.88 (0.02)[a]	0.48 (0.51)[a]
Education (years)	12.9 ± 2.5	14.6 ± 1.8	**0.0054 (–2.78)**	15.4 ± 2.5	15.4 ± 2.4	0.88 (–0.15)	**3.50×10^{-4} (–3.58)**
Estimated premorbid IQ	97.3 ± 9.0	101.0 ± 9.4	0.15 (–1.43)	106.8 ± 7.6	105.9 ± 9.0	0.57 (–0.57)	**1.91×10^{-6} (–4.76)**
Handedness (rt./lt.)	23/2	29/1	0.45 (0.48)[a]	67/4	85/3	0.50 (0.46)[a]	0.75 (0.10)[a]
Gray matter volume (mm3)	693.0 ± 78.6	683.3 ± 81.3	0.51 (–0.66)	709.7 ± 68.4	709.8 ± 84.5	>0.99 (<0.01)	0.067 (–1.83)
CPZeq (mg/day)	648.8 ± 540.9	518.8 ± 502.8	0.30 (–1.04)	–	–	–	–
Age at onset (years)	24.2 ± 11.2	23.9 ± 8.7	0.67 (–0.42)	–	–	–	–
Duration of illness (years)	11.1 ± 6.0	12.6 ± 9.9	0.87 (–0.16)	–	–	–	–
PANSS positive symptoms	16.6 ± 6.9	18.5 ± 6.4	0.24 (–1.17)	–	–	–	–
PANSS negative symptoms	19.4 ± 8.5	18.2 ± 6.3	0.79 (–0.26)	–	–	–	–
PANSS general psychopathology	35.8 ± 12.2	38.5 ± 10.4	0.32 (–1.00)	–	–	–	–

Means ± SD and *P* values are shown. [a]χ^2-test. PANSS, Positive and Negative Syndrome Scale; CPZ-eq, chlorpromazine equivalent of total antipsychotics. Complete demographic information was not obtained for all subjects (estimated premorbid IQ and PANSS: A carriers in patients, $N = 29$; estimated premorbid IQ: A carriers, $N = 87$ in controls).

healthy subjects. The subjects met criteria as follows: all subjects were biologically unrelated Japanese individuals and were recruited at Osaka University. The subjects were excluded from the analyses if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active cancer, cerebrovascular disease, epilepsy or seizures. Cases were recruited from both outpatients and inpatients at the university hospital. Each subject with schizophrenia had been diagnosed by at least two trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), based on an unstructured clinical interview. Schizophrenics with comorbid substance-related disorders or mental retardation were excluded. Controls were recruited through local advertisements. Psychiatrically, medically and neurologically healthy controls were evaluated using the structured clinical interview for DSM-IV-Non-Patient to exclude individuals who had current or past contact with psychiatric services. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University.

SNP selection and genotyping

A genetic variant, rs2494732 (SNP5), in the *AKT1* gene was selected for this study, as described in the introduction. The designation of the SNP in parenthesis was according to original study (Emamian et al. 2004). Venous blood was collected from the subjects and genomic DNA was extracted from whole blood according to standard procedures. Genotyping of SNP5 was carried out using TaqMan assays as previously described (Hashimoto et al. 2006, 2007). Detailed information on the PCR conditions and the primer pairs is available upon request. No deviation from Hardy-Weinberg equilibrium in the examined SNP was detected in the patients or controls ($P > 0.05$) (patients with schizophrenia; G/G: 56; A/G: 52; A/A: 9, healthy controls; G/G: 86; A/G: 78; A/A: 25). Because of the small number of subjects homozygous for the A allele, the A/A and A/G genotype groups were combined and treated as the A carriers for this study.

Cognitive measures

A full version of the WMS-R (Sugishita 2001; Wechsler 1987), a measure that is generally used to

measure memory and attentional functions, was administered to the subjects. The five indices, attention/concentration, verbal memory, visual memory, delayed recall and general memory, of the WMS-R were used to the analysis. The scores of indices were corrected by age. To assess sustained attention and vigilance, we used the CPT-IP. Compared to the simple version CPT-X, the CPT-IP is considered to be highly attention demanding (Cornblatt et al. 1988). This version is a prominent measure of attention in schizophrenia research (Cornblatt et al. 1989; Cornblatt and Keilp 1994). The CPT-IP was presented in a computerized version. The stimuli were two-, three-, or four-digit numbers in separate conditions. We performed each condition within a 1-min interval in order of two-, three-, or four-digit numbers. Each condition contained 150 stimuli. Each stimulus appeared on the monitor for 50 ms, followed by a dark time of 950 ms, for a total trial time of 1 s. A 150-trial condition, therefore, takes approximately 2.5 min to administer. Subjects were asked to respond (via a finger lift from a reaction-time key) whenever the same digit numeral appeared twice in succession during the sequence. In each condition, 20% of the stimuli are target pairs that are exactly alike and require a response; 20% are "catch" trials (pairs that are almost alike but are not quite identical); and 60% are randomly organized fillers. The parameter *D'* is used as outcome variable which is a measure of sensitivity composed of hits and false alarms. *D'2*, *D'3*, and *D'4* correspond to the number of digits in each number. *D'3* is more difficult than *D'2*, and *D'4* is the most difficult condition among the three different conditions.

Magnetic resonance imaging procedure

All MR studies were performed on a 1.5 T GE Sigma EXCITE system. A three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of 124 sagittal sections using a spoiled gradient recalled acquisition in the steady state (SPGR) sequence (TE/TR, 4.2/12.6 ms; flip angle, 15°; acquisition matrix, 256 × 256; 1NEX, FOV, 24 × 24 cm; slice thickness, 1.4 mm). Statistical analyses were performed with Statistical Parametric Mapping 5 (SPM5) software (http//www.fil.ion.ucl.ac.uk/spm) running on MATLAB R2007a (MathWorks, Natick, MA). MR images were processed using optimized voxel-based morphometry (VBM) in SPM5 according to VBM5.1-Manual (http://dbm.neuro.uni-jena.de/vbm/vbm5-for-spm5/manual/) as previously described in detail (Ashburner and Friston 2000; Good et al. 2001). We screened all scans and found no gross abnormalities such as infarct, haemorrhage and brain tumours in any of the subjects. Each image was visually confirmed to eliminate images with motion or metal artifacts, and then the anterior commissure-posterior commissure line was adjusted. The normalized segmented images were modulated by multiplication with Jacobian determinants of the spatial normalization function to encode the deformation field for each subject as tissue volume changes in the normal space. Finally, images were smoothed with a 12-mm full-width half-maximum of isotropic Gaussian kernel.

We first performed an exploratory whole brain search to investigate the effects of diagnosis, genotype and their interaction on gray matter volume in patients with schizophrenia and controls. These effects on gray matter volume were assessed statistically using the full factorial model for a 2 × 2 ANOVA in SPM5. We contrasted gray matter volumes between the genotype groups (smaller volume region in A carriers relative to individuals with G/G genotype, and larger volume region in A carriers relative to those with G/G genotype), the diagnosis groups (smaller volume region in patients with schizophrenia relative to controls) and their interaction. Age, sex and education years were included to control for confounding variables in the analysis. Since it is desirable to adjust for each subject's global gray matter volume (Good et al. 2001), adjustment was performed by entering the global gray matter values as a covariate. Non-sphericity estimation was used. The exploratory whole brain analysis yielded statistical parametric maps {SPM (t)} based on a voxel-level height threshold of $P < 0.001$ (uncorrected for multiple comparisons). To reduce the type I error due to small clusters, only clusters of more than 180 contiguous voxels were considered in the analysis. Given the a priori hypothesis, small volume correction (SVC) was applied to protect against type I error using family wise error (FWE). The significance level was set as $P < 0.05$ (*FWE* corrected) after SVC for spheres with a radius of 10 mm around the peak. Based on the priori hypothesis, we next performed volume-of-interest (VOI) approach to further compare significant regions of diagnosis–genotype interaction in the exploratory whole brain analysis. We extracted a sphere of 10 mm VOI radius from regions of interest because SVC applied for spheres with a radius of 10 mm around the peak. Anatomic localization was performed according to both MNI coordinates and Talairach coordinates, which were obtained from M. Brett's transformations (http://www.mrccbu.cam.ac.uk/Imaging/Common/mnispace.shtml) and presented as Talairach coordinates.

Statistical analysis

Statistical analyses were performed using PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls or between genotypes were analyzed using χ^2-tests for categorical variables and the Mann–Whitney *U*-test for continuous variables. The presence of Hardy–Weinberg equilibrium was examined using the χ^2-test for goodness of fit. Multivariate analysis of covariance (MANCOVA) is an extension of ANCOVA methods to cover cases where there is more than one dependent variable and where the dependent variables cannot simply be combined and are correlated with each other. The effects of the diagnosis, genotype and their interaction on memory performance in the WMS-R and attentional performance in the CPT were analyzed by two-way MANCOVA. Diagnosis and genotype statuses were included in the model as independent variables. Each WMS-R subscale score (attention/concentration, verbal memory, visual memory, delayed recall and general memory) on memory performance or each *D'* (*D'2*, *D'3* and *D'4*) on attentional performance was included as dependent variables. Complete demographic information about estimated premorbid IQ was not obtained for all subjects. As years of education are correlated with the premorbid IQ, we used the education years in place of the premorbid IQ as covariates. For the WMS-R, sex and years of education were treated as covariates, as they were possible confounding factors. For the CPT, sex, age, and years of education were treated as covariates. Post hoc analyses were performed as analyses of covariance (ANCOVA). Pearson's correlation coefficients were used to assess relationships between neurocognitive domains. We extracted relative gray matter volume as the "*y*" values from maxima voxel in the region of interest, and used these values in the VOI analysis using PASW. The effects of the variation in *AKT1* on the extracted VOI were tested by analyses of variance (ANOVA) without covariates, as the extraction of VOI was performed after confounding factors including age, sex, education years and total gray matter volumes were included in the whole brain search analysis. Statistical significance was defined as $P<0.05$.

Results

Association between a genetic variant in AKT1 and memory performances measured by the WMS-R

We examined the possible impact of SNP5 on memory performance measured by the WMS-R in 94 patients with schizophrenia and 121 healthy subjects. There was no difference in demographic variables between *AKT1* genotype groups (Table I). A two-way MANCOVA revealed significant effects of diagnosis ($F_{1,205}=28.55$, $P=6.35\times10^{-22}$) and marginally significant effects of genotype ($F_{1,205}=2.22$, $P=0.054$) on memory performance (Table IV and Figure 1). No genotype–diagnosis interaction was found ($P=0.52$). As expected, memory performance in patients with schizophrenia was poorer than in controls. Memory performance in A-carriers was poorer than in subjects homozygous for the G-allele. Post hoc two-way ANCOVA revealed significant effects of diagnosis (attention/concentration: $F_{1,209}=44.97$, $P=1.84\times10^{-10}$, verbal memory: $F_{1,209}=120.28$, $P=2.12\times10^{-22}$, visual memory: $F_{1,209}=53.39$, $P=5.69\times10^{-12}$, delayed recall: $F_{1,209}=130.70$, $P=7.97\times10^{-24}$ and general memory: $F_{1,209}=127.57$ $P=2.11\times10^{-23}$) and genotype (attention/concentration: $F_{1,209}=5.01$, $P=0.026$ and delayed recall: $F_{1,209}=3.99$, $P=0.047$). No genotype–diagnosis interaction was found ($P>0.39$). Genotype effects were found in the scores of attention/concentration and delayed recall (Figure 1), while there was no association between the genetic variation of *AKT1* and the scores on the other three indices (Figure 1). The attention/concentration and delayed recall scores in A allele carriers were lower than in homozygous G subjects. These results suggest that the A allele carriers could have poorer memory performance, particularly

Table IV. Association of a genetic variant of *AKT1* with the five indices of WMS-R.

	Schizophrenia ($N=94$)		Control ($N=121$)		*P* values ($F_{1,209}$ values)		
	G/G ($N=46$)	A carriers ($N=48$)	G/G ($N=57$)	A carriers ($N=64$)	Diagnosis effect	Genotype effect	Interaction
MANCOVA					**6.35×10^{-22} (28.55)**	0.054 (2.22)	0.52 (0.84)
Attention/concentration	94.3 ± 14.0	89.6 ± 17.0	110.0 ± 15.7	106.0 ± 13.1	**1.84×10^{-10} (44.97)**	**0.026 (5.01)**	0.85 (0.04)
Verbal memory	85.0 ± 17.9	85.1 ± 18.2	111.5 ± 15.0	110.6 ± 10.9	**2.12×10^{-22} (120.28)**	0.59 (0.29)	0.85 (0.03)
Visual memory	91.5 ± 19.7	90.3 ± 20.8	108.8 ± 11.4	109.1 ± 9.0	**5.69×10^{-12} (53.39)**	0.54 (0.38)	0.68 (0.17)
Delayed recall	85.0 ± 19.2	79.7 ± 20.0	112.0 ± 14.2	110.3 ± 12.4	**7.97×10^{-24} (130.70)**	**0.047 (3.99)**	0.39 (0.76)
General memory	85.0 ± 19.2	84.3 ± 19.6	113.1 ± 14.5	111.4 ± 10.1	**2.11×10^{-23} (127.57)**	0.35 (0.86)	0.86 (0.03)

MANCOVA, multivariate analysis of covariance. Means ± SD and *P* values are shown. Significant *P* values are indicated in bold.

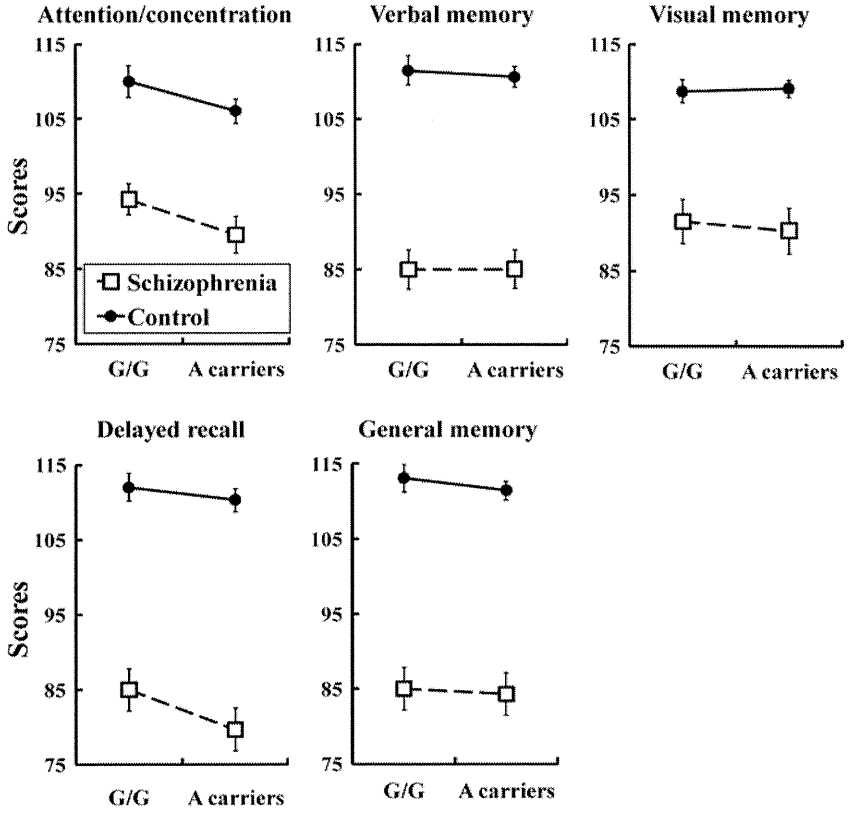

Figure 1. Association between the genetic variant of *AKT1* and memory performance measured by the Wechsler Memory Scale-Revised (WMS-R). The memory performance was measured using five domain scores, i.e. attention/concentration, verbal memory, visual memory, delayed recall, and general memory, of the WMS-R. Significant effects of diagnosis and genotype were observed. No diagnosis-genotype interaction was found. Memory performance in patients with schizophrenia was lower than in healthy subjects. A carriers showed lower memory performance compared to individuals with the G/G genotype. Error bars represent the standard error.

attention/concentration performance, as compared with the homozygous G subjects.

Association between the genetic variant of AKT1 and attentional performance measured by the CPT

The attention/concentration index of the WMS-R measures a complex attentional function comprising three subscales of mental control, digit span and visual memory span (Wechsler 1987), while the CPT-IP assesses vigilance/sustained attention in the attentional function. To evaluate the association between SNP5 and attentional function using a different assessment tool for attention, we examined the association using CPT-IP. There was no difference in demographic variables between *AKT1* genotype groups (Table II). The attention/concentration index of the WMS-R had significantly positive correlations with the attentional indices measured by the CPT in healthy sub-samples with an overlap (*D'2*: $r = 0.24$, df = 119, $P = 0.0068$, *D'3*: $r = 0.35$, df = 119, $P = 8.23 \times 10^{-5}$, and *D'4*: $r = 0.35$, df = 119, $P = 1.02 \times 10^{-4}$). Because *D'2*, *D'3* and *D'4* of the CPT cannot simply be combined and were correlated with each other, each *D'* was included in the MANCOVA analysis as dependent variables, and diagnosis and genotype statuses were included in the analysis as independent variables. Two-way MANCOVA revealed significant effects of diagnosis ($F_{1,172} = 11.44$, $P = 7.11 \times 10^{-7}$), genotype ($F_{1,172} = 3.71$, $P = 0.013$) and their interaction ($F_{1,172} = 4.01$, $P = 0.0086$) on attentional performance (Table V and Figure 2). As a genotype–diagnosis interaction was found, we analyzed the genotype effect on attentional performance in patients and controls separately. The attentional performance of the A carriers was significantly lower than that of homozygous G subjects in patients with schizophrenia ($F_{1,53} = 4.49$, $P = 0.0070$), while there was no significant genotype effect in the healthy controls ($P = 0.38$). Post hoc two-way ANCOVA revealed significant effects of diagnosis (*D'2*: $F_{1,174} = 15.35$, $P = 1.28 \times 10^{-4}$, *D'3*: $F_{1,174} = 28.01$, $P = 3.60 \times 10^{-7}$, and *D'4*: $F_{1,174} = 29.23$, $P = 2.10 \times 10^{-7}$), genotype (*D'2*: $F_{1,174} = 8.99$, $P = 0.0031$ and *D'3*: $F_{1,174} = 4.76$, $P = 0.030$) and their interaction (*D'2*: $F_{1,174} = 11.14$, $P = 0.0010$) (Figure 2). All three scores in patients with schizophrenia were significantly lower than those in healthy controls. A carriers had lower

Table V. Association of a genetic variant of *AKT1* with the scores of CPT.

	Schizophrenia ($N=60$)		Control ($N=121$)		P values ($F_{1,174}$ values)		
	G/G ($N=35$)	A carriers ($N=25$)	G/G ($N=57$)	A carriers ($N=64$)	Diagnosis effect	Genotype effect	Interaction
MANCOVA					**7.11×10^{-7} (11.44)**	**0.013 (3.71)**	**0.0086 (4.01)**
D'2	3.54 ± 0.67	3.00 ± 0.74	3.67 ± 0.47	3.70 ± 0.47	**1.28×10^{-4} (15.35)**	**0.0031 (8.99)**	**0.0010 (11.14)**
D'3	2.87 ± 0.85	2.51 ± 0.82	3.46 ± 0.67	3.31 ± 0.65	**3.60×10^{-7} (28.01)**	**0.030 (4.76)**	0.36 (0.86)
D'4	1.56 ± 0.96	1.33 ± 0.76	2.31 ± 0.90	2.30 ± 0.87	**2.10×10^{-7} (29.23)**	0.47 (0.53)	0.47 (0.53)

MANCOVA: multivariate analysis of covariance. Means ± SD and P values are shown. Significant P values are indicated in bold.

scores for *D'2* and *D'3* than homozygous G subjects (Figure 2). As a genotype–diagnosis interaction was found only in *D'2*, we analyzed the effects of genotype on *D'2* score in patients and controls separately. The *D'2* score of the A carriers was significantly lower than that of homozygous G subjects in patients with schizophrenia ($F_{1,55}=13.51$, $P=5.39\times10^{-4}$); by contrast, there was no significant genotype effect in the healthy controls ($F_{1,116}=0.24$, $P=0.63$) (Figure 2). No interaction was found for *D'3* or *D'4* ($P=0.36$, 0.47, respectively). The *D'2* score of the A carriers was still significantly lower than the score of homozygous G subjects in patients with schizophrenia even after adjusting scores of positive and negative symptoms in PANSS and the chlorpromazine equivalent of total antipsychotics as covariates ($F_{1,52}=14.09$, $P=4.41\times10^{-4}$). These results suggest that variation in the *AKT1* gene could be related to deficits of performance in sustained attention and vigilance of attention in patients with schizophrenia.

Effect of the AKT1 polymorphism on brain structure

We first performed an exploratory whole brain analysis to investigate the effects of diagnosis, genotype and their interaction on gray matter volumes. The only difference in demographic variables between the *AKT1* genotype groups was for years of education in patients (Table III). We found significant effects of diagnosis and diagnosis–genotype interaction (uncorrected $P<0.001$), while we did not find any genotype effect for all subjects (uncorrected $P>0.001$). Patients with schizophrenia showed smaller gray matter volumes compared with controls, mainly in the frontal lobe and the temporal lobe (data not shown), which was consistent with previous studies (Chan et al. 2009; Ellison-Wright and Bullmore 2010). Significant diagnosis–genotype interactions were gray matter volumes in the right inferior parietal lobule, the right superior frontal gyrus, the left superior temporal gyrus and the left caudate (Table VI).

It has been suggested that three regions, the right inferior parietal lobule, the thalamus and the anterior cingulate gyrus, are involved in attentional processes (Salgado-Pineda et al. 2004). These three regions have been smaller volumes in patients with schizophrenia compared with controls and have been associated with functional dificits of attention during CPT-IP. In addition, *AKT1* SNP4 has been associated with reduced gray matter volumes in the bilateral

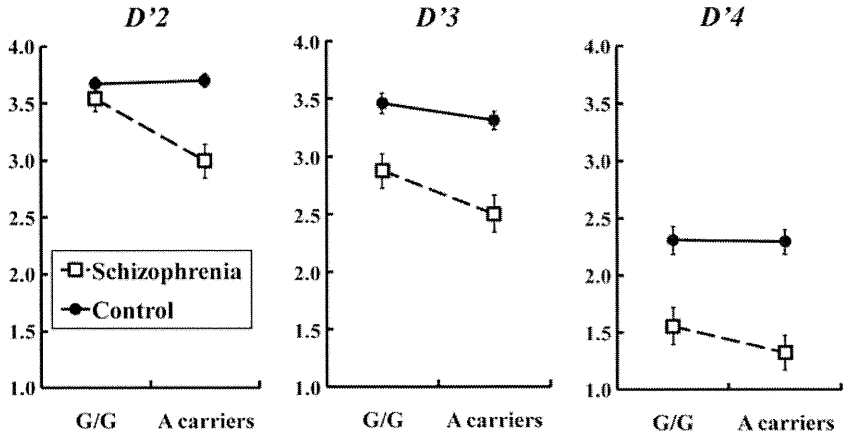

Figure 2. Association between the genetic variant of *AKT1* and attentional performance measured by the continuous performance test (CPT). Attentional performance was measured by three different conditions, i.e. *D'2*, *D'3*, and *D'4*, of the CPT. Significant effects of diagnosis, genotype and their interaction were observed. The genotype effect was observed in patients with schizophrenia, but not in healthy subjects. Patients with schizophrenia showed lower attentional performance than healthy subjects. Patients with A allele showed lower attentional performance than patients with G/G genotype. Error bars represent the standard error.

Table VI. Regions of the diagnosis-*AKT1* genotype interaction on brain morphology in an exploratory whole brain analysis (uncorrected $P<0.001$, cluster size >180).

Brain regions	R/L	BA	Cluster Size	T_{206} values	Talairach coordinates		
					x	*y*	*z*
AKT1 genotype–diagnosis interaction							
Frontal lobe							
Superior frontal gyrus	R	11	249	4.27	11	49	−18
Temporal lobe							
Superior temporal gyrus	L	41	390	4.00	−51	−20	3
Superior temporal gyrus	L	22	408	3.64	−54	−37	13
Parietal lobe							
Inferior parietal lobule	R	40	193	4.06	55	−27	35
Subcortical							
Caudate	L	–	211	3.71	−11	3	5

R, right; L, left; BA, Brodmann area.

caudate and right prefrontal cortex (Tan et al. 2008b) and SNP2 has been associated with decreased gray matter density in the medial, dorsolateral, and inferior prefrontal cortex (Pietilainen et al. 2009). Thus, we hypothesized that the variation in *AKT1* could be associated three regions associated with attentional function or/and the caudate and prefrontal cortex. Based on the priori hypothesis, we applied *FWE* correction to gray matter regions of interest in the right inferior parietal lobule (*FWE*-corrected $P=0.006$, Figure 3A), the right superior frontal gyrus (*FWE*-corrected $P=0.003$, Figure 3B), and the left caudate (*FWE*-corrected $P=0.017$, Figure 3C).

We next focused on the three regions of diagnosis–genotype interaction based on the priori hypothesis. Two-way ANOVA revealed significant diagnosis–genotype interactions in the extracted region from the center of the right inferior parietal lobule (55, −27, 35; $F_{1,210}=14.63$, $P=1.72\times10^{-4}$), the right superior frontal gyrus (11, 49, −18; $F_{1,210}=16.40$, $P=7.21\times10^{-5}$) and the left caudate (−11, 3, 5; $F_{1,210}=11.44$, $P=8.56\times10^{-4}$). As the genotype–diagnosis interactions were found, we analyzed the effects of genotype on these regions in patients and controls separately (Figure 3D–F). In patients with schizophrenia, A carriers had smaller gray matter volumes in these regions than homozygous G subjects, respectively (the right inferior parietal lobule; $F_{1,53}=12.26$, $P=9.46\times10^{-4}$, the right superior frontal gyrus; $F_{1,53}=16.19$, $P=1.83\times10^{-4}$ and the left caudate; $F_{1,53}=8.69$, $P=0.0048$). In contrast, in controls, homozygous G subjects had marginally smaller gray matter volumes in these regions than A carriers (the right inferior parietal lobule; $F_{1,157}=5.45$, $P=0.021$, the right superior frontal gyrus; $F_{1,157}=3.88$, $P=0.051$ and the left caudate; $F_{1,157}=3.49$, $P=0.064$). Even if handedness, which was a confounding factor, was co-varied in these VOI analyses, these effects of the variation in *AKT1* on the extracted VOI remained significant. These data suggest that *AKT1* SNP5 might be associated with several brain morphological vulnerabilities in patients with schizophrenia, and that the smaller volume of the right inferior parietal lobule might be associated with structural vulnerability for attentional performance in schizophrenia, as the volume of the inferior parietal lobule has been positively correlated with attentional performance in patients with schizophrenia (Salgado-Pineda et al. 2004).

Discussion

We evaluated the association between SNP5 in *AKT1* and attentional performance and brain structure in a Japanese population. We examined this particular polymorphism after it was associated with schizophrenia in Asian populations in a recent meta-analysis (Shi et al. 2008) and after it was shown to predict treatment response to risperidone in Japanese patients with schizophrenia (Ikeda et al. 2008). We found that A carriers of SNP5, which is the risk allele for schizophrenia in Asian populations, performed worse on attentional performance, a capacity that is impaired in patients with schizophrenia compared with healthy subjects, than subjects with the G/G genotype as determined by WMS-R and CPT. In the CPT, the poorer performance in A carriers was found in the *D'2* and the *D'3*. In the *D'2* condition, the poorer attentional performance in A carriers was found only in the patients with schizophrenia, but not in controls. There was no association between the genotype and performance in the *D'4* condition. The difficulty of the three processing-load conditions (*D'2*, *D'3*, and *D'4*) is different, with the *D'2* condition being simpler than the others, suggesting that the lower CPT load (*D'2*) might be associated with a simpler attentional function than the higher CPT loads (*D'3* and *D'4*). It is expected to be worsening

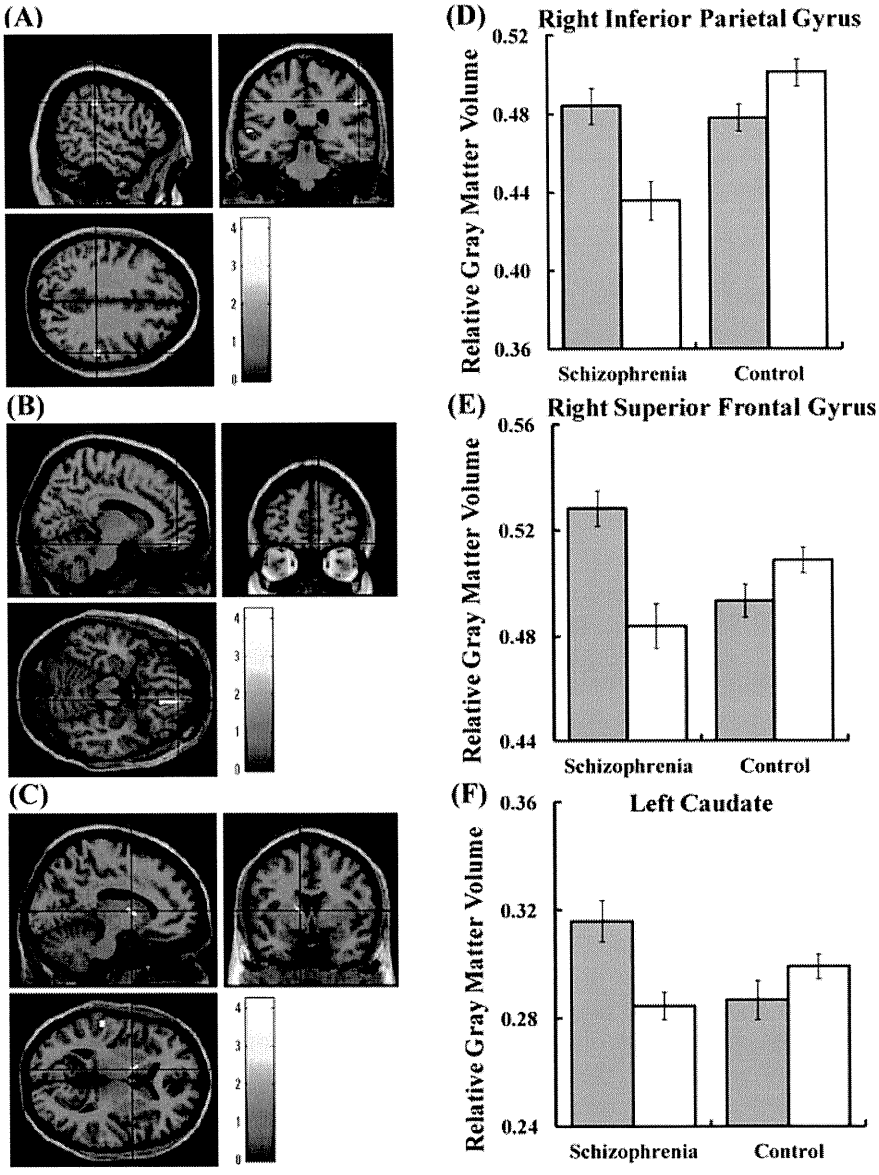

Figure 3. Impact of diagnosis-*AKT1* genotype interaction on gray matter volumes. (**A–C**) Anatomical localizations are displayed on coronal, sagittal, and axial sections of a normal MRI spatially normalized into the Montreal Neurological Institute template (uncorrected $P<0.001$, cluster size >180). Significant clusters of diagnosis–genotype interaction was in the right inferior parietal lobule (Talairach coordinates; 55, –27, 35, **A**), in the right superior frontal gyrus (11, 49, –18, **B**) and in the left caudate (–11, 3, 5, **C**). The regions are shown as cross-hairline. The colour bars show *t* values corresponding to the colour in the figures. (**D–F**) Each column shows relative gray matter volumes extracted from the right inferior parietal lobule (55, –27, 35, **D**), right superior frontal gyrus (11, 49, –18, **E**) and left caudate (–11, 3, 5, **F**). Gray bars represent individuals with G/G genotype, while white bars represent A carriers. Error bars represent the standard error.

performance with increasing task difficulty and to increase the difference of the performance between cases and controls. The reason why the *AKT1* genotype was not associated with the *D'4* condition might be considered a floor effect in the CPT condition because of difficulty to achieve the *D'4* condition in patients and controls. On the other hand, the reason why the genotype was not associated with the *D'2* in controls might be considered a ceiling effect in the CPT condition because of ease to achieve the *D'2* condition in healthy subjects. The attention/concentration index of the WMS-R assesses a complex attentional function comprising three subscales of mental control, digit span and visual memory span (Wechsler 1987). The correlations of the attention/concentration of the WMS-R with *D'2* ($r=0.24$), *D'3* ($r=0.35$) or *D'4* ($r=0.35$) were modest. These results require a careful interpretation because attentional functions in these measures may imply different cognitive processes. These results suggest that A carrier might have deficits in attentional performance and that the attentional deficits in A carrier might be detected by easier tasks in patients with schizophrenia.

We found that the gray matter volume of the right inferior parietal lobule in carriers of the A allele was significantly smaller than that in homozygous G subjects with schizophrenia. There was no genotype effect on the gray matter volumes in two other regions, the thalamus and the anterior cingulate gyrus, in patients with schizophrenia (data not shown), although it has been reported that these regions are also related to attentional function (Salgado-Pineda et al. 2004). The right inferior parietal lobule have been smaller volumes in patients with schizophrenia compared with controls and have been associated with functional dificits of attention during CPT-IP (Salgado-Pineda et al. 2004). The previous study measured stimuli of one-digit numbers as CPT-IP performance (*D'1*). The *D'1* is simpler condition than the *D'2*, although we did not measure stimuli of one-digit numbers. These data suggest that variation of the *AKT1* gene might be associated with the gray matter volume of the right inferior parietal lobule with structural vulnerability of simple attentional performance in schizophrenia. To reinforce assumption of relevance of inferior parietal lobule for attentional processing and to investigate potential genotype effects on such relationship, we performed a preliminary investigation for gray matter volume–behavioural correlations in the overlapping sub-samples. Although the gray matter volume of the right inferior parietal lobule had marginal positive correlations with attentional performances, the correlations did not reach statistical significance due to the small sample size (data not shown). Since the analyses of the associations between *AKT1* and memory, attention and gray matter volume were performed on different sub-samples with insufficient overlap, our results could not be clearly interpreted as the findings linking *AKT1*, the inferior parietal lobule and attentional dysfunction into one multimodel gene-morphology-cognition vulnerability factor. It would be desirable to perform all of the association analyses between *AKT1*, neurocognitive measures and brain images on the same subjects of age and sex matched case–control. Further studies are needed to elucidate the link on such subjects.

We also found relationships between *AKT1* SNP5 and gray matter volumes in the right superior frontal gyrus and the left caudate. These regions have been associated with different SNPs in *AKT1* gene (Pietilainen et al. 2009; Tan et al. 2008b). Interestingly, three independent studies showed evidence for a role of *AKT1* in the brain morphology. Frontostriatal circuits are related with cortical dopaminergic function and implicated in schizophrenia (Pantelis et al. 1997). Consistent with the preclinical evidence that couples AKT1 to dopaminergic frontostriatal function and cellular structure (Beaulieu et al. 2004, 2005; Emamian et al. 2004), human genetic variation in *AKT1* has been associated with cognitive performances referable to the frontostriatal dopaminergic system (Tan et al. 2008b). Brain networks between frontal cortex and striatum are implicated in the cognitive functions reflecting executive function, IQ, and processing speed (Alexander et al. 1986; Pantelis et al. 1997; Tan et al. 2008b). Although we did not evaluate these cognitive functions in this study, *AKT1* SNP5 might also be associated with these cognitive functions.

In these VOI approaches, A carriers had smaller gray matter volumes than homozygous G subjects in patients, but there was a trend in the opposite direction in healthy subjects. This finding can be interpreted in two ways. First, it is possible that the genetic variant is related in a different way to dysregulated levels of AKT1 protein or dopamine between patients and controls. Second, because we applied three multiple comparisons in the VOI analyses, there is the possibility of type 1 errors in healthy controls (the right inferior parietal lobule; corrected $P = 0.063$, the right superior frontal gyrus; corrected $P = 0.15$ and the left caudate; corrected $P = 0.19$). Both these explanations are speculative and have to be treated with caution.

Since *Akt1*-knockout mice showed deficits in hippocampal neurogenesis and behavioural effects associated with the hippocampus (Balu et al. 2011), hippocampus might also be a structure associated with attentional performance and memory. However, we did not find genotype effect on the hippocampus in the present study. It remains unclear whether *AKT1* is related to hippocampal volume in patients with schizophrenia, and thus further investigation is needed.

The present study is the first report demonstrating an association between SNP5 in the *AKT1* gene and neuropsychological parameters and gray matter volume in an Asian population. Four studies have investigated an association between SNPs in *AKT1* and learning, memory or attention in Caucasian populations (Pinheiro et al. 2007; Tan et al. 2008b; Pietilainen et al. 2009; Blasi et al. 2011). In subjects with European ancestry, there was an association between haplotypes comprising SNP3, SNP4 and SNP5 as well as the single SNP4 in *AKT1* and a factor comprised of IQ and processing speed; however, associations of the same SNP with six other cognitive factors, including verbal memory, working memory, visual memory, Wisconsin card sort, attention or digit span, were not found (Tan et al. 2008b). In healthy subjects, there was an association between SNP4 in *AKT1* and correct responses in the CPT (Blasi et al. 2011). On the other hand, there was no significant association between any of the five SNPs (rs3803300 (SNP1), SNP2, SNP3, SNP4 and SNP5) in *AKT1* and five domain scores (processing

speed, reasoning, verbal memory, working memory, and vigilance) in patients with schizophrenia (Pinheiro et al. 2007). Moreover, an association of SNP2 with verbal learning and memory was found in Finnish twins (Pietilainen et al. 2009). However, no association of SNP4 or SNP5 with any neuropsychological parameters has been shown. These inconsistencies might be effects of mixed populations or mixed subjects, since Pinheiro et al. analyzed the associations in subjects of African, European, and other ethnic populations and Pietilainen et al. analyzed the associations in combined samples of twin pairs of healthy controls, patients with schizophrenia, and patients with bipolar disorder. Taken together, these data suggest that SNPs in the *AKT1* gene, which is associated with schizophrenia, might exert different genotype effects on neuropsychological parameters in different ethnic populations.

Previous reports have indicated that lithium or valproate target AKT1 (Gupta et al. 2011). In the present study, there were only three patients taking lithium (G/G $N=2$, mean dose $\pm$ SD (mg/day): 600.0 ± 0.0, A carriers $N=1$, 400.0) and six patients taking valproate (G/G $N=4$, 800.0 ± 163.3, A carriers $N=2$, 400.0 ± 282.3). There was no significant difference of memory, attention or gray matter volume between patients with and without these drugs treatment (data not shown), suggesting that these drugs might not affect our findings.

The D2/AKT1/GSK-3β signalling pathway has been involved in the downstream intracellular effects of dopamine. Dopaminergic dysregulation plays an important role in the pathophysiology of schizophrenia. Different molecular pathways as downstream of dopamine D2 receptors have been identified: the classic cAMP-PKA pathway and the cAMP-independent D2 signalling cascade pathway that includes the AKT1, which phosphorylates to inhibit another protein kinase, GSK3β (Freyberg et al. 2010). D2 stimulation by dopamine inhibits AKT1 signalling through dephosphorylation via the β-arrestin2/phosphatase PP2A complex (Beaulieu et al. 2007a,b), indicating the specific relationship between D2 receptor signalling and AKT1. In the *Akt1*-deficient mice, impaired working memory and prepulse inhibition of startle were caused by D2 agonists (Emamian et al. 2004; Lai et al. 2006). D2 receptors are essential for the Akt inhibition by dopamine (Beaulieu et al. 2007b). Importantly, reduced protein expression of AKT1 and phosphorylation of GSK3β have been indicated in postmortem brains (prefrontal cortex) and lymphocytes of patients with schizophrenia (Emamian et al. 2004; Thiselton et al. 2008). Furthermore, a D2 antagonist antipsychotic increases AKT1 and GSK-3β phosphorylation (Kang et al. 2004). Combined with the earlier involvement of D2 signaling in attentional processing (Zhang et al. 2007) and the relationship between *AKT1* and attentional function in this study, the D2/AKT1/GSK-3β signalling pathway might be involved in the pathophysiology of attentional deficits in schizophrenia.

There were several limitations to this study. After confirmed that the patients' symptoms were stable and doses of their antipsychotic treatments were maintained over 4 weeks, we performed all tests in patients with schizophrenia. However, we did not exclude the possibility that treatment status affect subtle genetic effects in patients with schizophrenia. To reduce the type I error due to small clusters, we applied only clusters of more than 180 contiguous voxels in the neuroimaging analysis. Although we have empirically selected the cluster size threshold of 180 contiguous voxels, it should be noted that the extent to which the type I error is reduced is unknown. A whole brain *FWE* correction was not used in the analysis. SVC for a radius of 10 mm around the peak applied in this study was liberal. These could lead to a type I error in this study. It is unclear whether SNP5 is associated with biological activity on AKT signalling, such as AKT1 protein levels and phosphorylation of GSK3β. The lack of such association makes it unclear if our results are directly linked with *AKT1* rs2494732 or with other polymorphisms in linkage disequilibrium with this genetic variant. Further study to elucidate functional molecular effects of SNP5 is required.

In conclusion, the *AKT1* polymorphism could be related to the deficits in simple performance of the attentional tasks and the gray matter volumes of the right inferior parietal lobule and the frontostriatal region in Japanese patients with schizophrenia. Further studies are needed to elucidate an underlying genetic vulnerability to neurobiological traits in schizophrenia.

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Statement of interest

None to declare.

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ORIGINAL INVESTIGATION

The *KCNH2* gene is associated with neurocognition and the risk of schizophrenia

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Abstract

Objectives. A genetic variant (rs3800779; M30) in the *KCNH2* gene has been associated with schizophrenia, a lower intelligence quotient (IQ) and processing speed scores, altered brain functions and increased *KCNH2-3.1.* mRNA levels in the hippocampus. The aims of this study were to investigate whether the *KCNH2* polymorphism is associated with schizophrenia-related neurocognitive deficits and to confirm the association between the variant and schizophrenia. *Methods.* The effects of the risk genotype on IQ and seven neurocognitive batteries were examined by the analysis of covariance in 191 healthy subjects. We performed a meta-analysis of the association between M30 and schizophrenia using five independent ethnic groups (1,720 cases; 2,418 controls). *Results.* Consistent with the previous study, we provided evidence that subjects with the risk T carriers had significantly lower IQ scores than those with the G/G genotype ($P = 0.048$). Of the seven neurocognitive batteries, subjects with the risk genotype demonstrated lower performances on attention/vigilance ($P = 0.0079$) and working memory ($P = 0.0066$) relative to subjects with the G/G genotype. Meta-analysis demonstrated evidence for an association between M30 and schizophrenia without showing heterogeneity across studies (odds ratio = 1.18; $P = 0.0017$). *Conclusions.* These data suggest that the *KCNH2* polymorphism could be associated with schizophrenia-related neuropsychological deficits and the risk of developing schizophrenia.

Key words: *schizophrenia, KCNH2 (potassium channel, voltage-gated subfamily H, member 2), intelligence quotient (IQ), single nucleotide polymorphism (SNP), meta-analysis, neurocognition*

Introduction

Schizophrenia is a common, complex psychiatric disease characterized by both clinical and genetic heterogeneity. There are strong genetic components of the disease with an estimated heritability of approximately 80% (Cardno and Gottesman 2000; Tsuang 2000). Attempts have been made to minimize this heterogeneity and to clarify the genetic architecture. One strategy for gene discovery proposes using quantitative neurobiological traits as intermediate phenotypes instead of relying on the diagnosis of schizophrenia alone to identify cases for investigation (Meyer-Lindenberg and Weinberger 2006; Tan et al. 2008a). This strategy has the potential to reduce clinical and genetic heterogeneity by applying alternative phenotypes that better reflect the underlying genetic vulnerability than does diagnostic categorization. Neurocognitive deficits, a core component of schizophrenia (Green 2006), are considered promising intermediate phenotypes for gene

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discovery in schizophrenia (Snitz et al. 2006; Husted et al. 2009). There is substantial evidence suggesting that most cognitive abilities have a genetic basis (Chen et al. 1998; Posthuma et al. 2001; Berrettini 2005; Husted et al. 2009). The estimated heritabilities of processing speed, attention/vigilance, verbal intelligence quotient (IQ) and performance IQ are 33–48, 48–62, 85 and 69%, respectively.

Recently, Huffaker et al. identified a potential schizophrenia susceptibility (human ether-a-go-go-related) gene, *KCNH2,* which encodes a voltage-activated potassium channel (Huffaker et al. 2009). The *KCNH2* gene contains 15 exons spanning 33 kb on chromosome 7q35–q36. A genetic variant (rs3800779; M30) in the *KCNH2* gene predicts lower IQ and processing speed scores, decreased hippocampal volume, altered memory-linked hippocampal functions and working memory-linked prefrontal functions. It also predicts increased expression levels of a primate- and brain-specific *KCNH2*-3.1 isoform in the hippocampus (Huffaker et al. 2009). Expression of *KCNH2*-3.1 in rodent cortical neurons causes a marked alteration in KCNH2 channel physiology resulting in high-frequency, nonadapting neuronal firing patterns (Huffaker et al. 2009). In this study, we examined the effects of the M30 genotype on IQ and seven neurocognitive functions shown to be associated with genetic liability in schizophrenia. We then conducted a meta-analysis of M30 in previously reported samples added to a Japanese sample to establish further evidence for an association between the *KCNH2* gene and schizophrenia.

Methods and materials

Subjects

Neurocognitive test data were available for 191 Japanese healthy individuals (49.2% males (94/97); mean age ± SD: 36.0 ± 11.5 years; years of education ± SD: 15.5 ± 2.4 years). Data from different number of subjects were available in each test (general IQ 143 subjects, speed of processing 188, attention/vigilance 191, working memory 190, Verbal Learning and Memory 190, Visual Learning and Memory 190, Reasoning and problem solving 150, and Social cognition 86). Demographic variables for subjects included in each cognitive test are shown in Supplementary Table I (available online). Although we attempted to examine all neurocognitive tests from all subjects as much as we could, all tests data were available for 83 subjects. Because an association between an SNP in the *KCNH2* gene and cognitive function was observed in healthy controls, we attempted to replicate the previous association finding in healthy controls (Huffaker et al. 2009). The use of healthy subjects to investigate an association between a genetic variant and neurocognitive function avoids the potential confounders related to the duration of illness and medical treatment. Healthy controls were recruited by local advertisements in Osaka, Japan. Psychiatrically, medically and neurologically healthy controls were evaluated using the Structured Clinical Interview for DSM-IV-Non-Patient Edition (SCID-I/NP) to exclude individuals who had received psychiatric medications. Subjects were also excluded from this study if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headaches, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active cancer, cerebrovascular disease, epilepsy, seizures, substance-related disorders or mental retardation. We excluded any control subjects with neurological disorders or first- or second-degree relatives with psychiatric disorders using an unstructured interview. All subjects were biologically unrelated Japanese individuals.

The subjects for the genetic association study consisted of 478 unrelated patients with schizophrenia (48.3% males (231/247); mean age ± SD: 48.4 ± 15.7 years) and 640 unrelated healthy controls (46.3% males (296/344); mean age ± SD: 58.9 ± 21.4 years). All subjects used in this analysis are unrelated Japanese, as described previously (Ohi et al. 2009b, 2010). Cases were recruited from both outpatients and inpatients at Osaka University Hospital and the psychiatric hospitals. Each subject with schizophrenia had been diagnosed by at least two trained psychiatrists based on an unstructured clinical interview; diagnoses were made based on the criteria of the DSM-IV. Controls were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals who had current or past contact with psychiatric services. 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 was approved by the Research Ethical Committee of Osaka University.

SNP selection and SNP genotyping

We selected rs3800779 (M30) in the *KCNH2* gene because this SNP has been associated with schizophrenia, as described in the introduction (Huffaker et al. 2009). Venous blood was collected from the subjects, and genomic DNA was extracted from whole blood according to standard procedures. The SNP was genotyped using the custom-designed

TaqMan 5'-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA, USA), as described previously (Hashimoto et al. 2007). No deviation from Hardy–Weinberg equilibrium in the examined SNP was detected in patients with schizophrenia or in controls ($P > 0.05$).

Neurocognitive testing

General intellectual function was derived from the Full Scale IQ portion of the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) (Wechsler 1997). The Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Neurocognition Committee selected seven neurocognitive domains from all available factor-analytic studies of cognitive performance in schizophrenia patients (Green et al. 2004; Nuechterlein et al. 2004). Seven neurocognitive batteries were selected based upon previous studies to assess the following seven domains (Nuechterlein et al. 2004, 2008): (1) speed of processing, (2) attention/vigilance, (3) working memory, (4) verbal learning and memory, (5) visual learning and memory, (6) reasoning and problem solving, and (7) social cognition. The speed of processing was assessed using the Category Fluency Test (total number of animals named in 60 s) (Sumiyoshi et al. 2004). Attention/vigilance was evaluated using the Continuous Performance Test-Identical Pairs version (*d'*) (Cornblatt et al. 1988). Working memory was measured using the Wechsler Memory Scale-Revised (WMS-R) digit span subtest (number of correct trials) (Sugishita 2001). Verbal learning and memory was assessed using the immediate recall portion of the Rey Auditory Verbal Learning Test (Lezak 1995) in which the participants were asked to recall a list of 15 words spoken by a tester. The procedure was repeated five times (sessions 1–5), and the sum of the recalled words from sessions 1 to 5 was used for the analysis. If the participants scored 15/15, we treated the scores of the participant as 15 after the session; possible scores range from 0 to 75. Visual learning and memory was evaluated using the visual reproduction I subtest of the WMS-R (number of correct trials) (Sugishita 2001). Reasoning and problem solving was measured using the tower of Hanoi task (number of correct trials) (Ohi et al. 2009a). Social cognition was assessed using the Emotion Recognition test (correct rate of the Facial Emotion Labeling Test (FELT)) (Sekiyama et al. 2008). The subjects included in this analysis were assessed by trained clinical psychologists to obtain scores on the WAIS-III Full Scale IQ and the seven schizophrenia-related neurocognitive batteries.

Meta-analysis

The studies included in the meta-analysis were obtained using PubMed using the search terms "KCNH2" and "schizophrenia". The analyzed data encompassed all publications up to October 2010. Additionally, references cited in the publications obtained were examined to identify additional potentially relevant studies that might not be listed in PubMed. Studies were included in the meta-analysis if they met the following criteria: (1) published in a peer-reviewed journal in English and (2) included a genetic association study between the *KCNH2* gene and schizophrenia. Our meta-analysis included allele frequency data from all available case–control studies only and did not include the original family-based dataset that provided strong evidence for the positive association in the original report by Huffaker et al. (Huffaker et al. 2009). We calculated each number of alleles from the allele frequency and the odds ratio data for each study.

Statistical analyses

Statistical analyses were performed using the PASW Statistics 18.0 software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls or between genotypes were analyzed using χ^2-tests for categorical variables and the Mann–Whitney *U*-test for continuous variables. Based on the assumption that demographic variables such as age and education years might not be fitted to a normal distribution, we used the nonparametric Mann–Whitney test arbitrary to assess the demographic variables. The presence of Hardy–Weinberg equilibrium was examined using the χ^2-test for goodness of fit. To control for confounding factors such as age, sex and years of education, we used a one-way analysis of covariance (ANCOVA) for neurocognitive tests, based on the assumption that the neurocognitive variables could be fitted to a normal distribution. The effect of the *KCNH2* genotype on IQ was analyzed by a one-way ANCOVA with sex and years of education as covariates because the IQ scores were already corrected for age. The effects on the seven neurocognitive domains were analyzed by a one-way ANCOVA with age, sex and years of education as covariates. Bonferroni correction was applied for multiple testing on seven domains to avoid type I errors. Standardized effect sizes were indicated using Cohen's *d* and η^2.

The meta-analysis was performed using the Comprehensive Meta-Analysis software (Version 2.0, BIOSTAT, Englewood Cliffs, NJ, USA). Cochran's χ^2-based *Q*-statistical test was performed to assess possible heterogeneity among studies. The fixed-effect

Table I. Impact of M30 in the *KCNH2* gene on IQ and on seven cognitive batteries.

Variables	Healthy subjects						ANCOVA		
	n	*n*	T carrier	*n*	G/G	Cohen's *d*	*F*	*P* values	η^2
General intellectual function	143	29	107.5 ± 15.4	114	110.3 ± 10.9	−0.21	3.98	0.048	0.028
Speed of processing	188	35	22.4 ± 5.4	153	21.2 ± 4.7	0.24	0.76	0.39	0.004
Attention/vigilance	191	36	3.63 ± 0.51	155	3.75 ± 0.47	−0.24	7.20	0.0079	0.037
Working memory	190	36	15.3 ± 3.9	154	16.8 ± 3.7	−0.39	7.55	0.0066	0.039
Verbal learning and memory	190	36	56.4 ± 9.0	154	57.9 ± 7.9	−0.17	2.02	0.16	0.011
Visual learning and memory	190	36	38.8 ± 2.9	154	39.3 ± 2.1	−0.20	2.49	0.12	0.013
Reasoning and problem solving	150	31	13.2 ± 7.5	119	13.8 ± 7.1	−0.08	0.95	0.33	0.006
Social cognition	86	19	58.8 ± 13.1	67	62.3 ± 13.1	−0.27	2.49	0.12	0.030

IQ, intelligence quotient; ANCOVA, analysis of covariance. Means ± SD are shown. The effect sizes are typically categorized as small ($d = 0.20$, $\eta^2 = 0.01$), medium ($d = 0.50$, $\eta^2 = 0.06$) or large ($d = 0.80$, $\eta^2 = 0.14$). To control for confounding factors, the effect of the *KCNH2* genotype on IQ was analyzed by one-way ANCOVA with sex and years of education as covariates because the IQ scores were already corrected for age. The effects on seven neurocognitive domains were analyzed by one-way ANCOVA with age, sex and years of education as covariates.

model described by Mantel-Haenszel was applied in the absence of heterogeneity ($p > 0.05$). The significance of the pooled odds ratio (OR) was assessed using a *z*-test. The significance level for all statistical tests was set at two-tailed $P < 0.05$.

Results

The effect of the KCNH2 risk polymorphism on IQ and on seven neurocognitive batteries

There were no differences in demographic variables – age, sex, or years of education – between genotype groups in each cognitive test (Supplementary Table I available online). As shown in Table I, we found a significant genotype effect on general intellectual function ($F_{1,139} = 3.98$, $P = 0.048$). Additionally, we found significant genotype effects on attention/vigilance ($F_{1,186} = 7.20$, $P = 0.0079$) and working memory ($F_{1,185} = 7.55$, $P = 0.0066$) from the seven batteries. The effect sizes (η^2) of IQ, attention/vigilance and working memory were 0.028, 0.037 and 0.039, respectively. Subjects with the risk T carriers had lower performances on these tests than did those with the G/G genotype (Figure 1). The genotype effect on working memory remained positive after the correction for multiple tests (corrected $P = 0.046$), while the genotype effect on attention/vigilance did not reach statistical significance after the correction (corrected $P = 0.055$). No significant genotype effect was found in any other cognitive batteries ($P > 0.05$).

Association between a genetic variant in the KCNH2 gene and schizophrenia by meta-analysis

The frequency of the T allele of M30 was higher in patients (11.0%) than in controls (9.7%) in the Japanese population used in this study. The direction

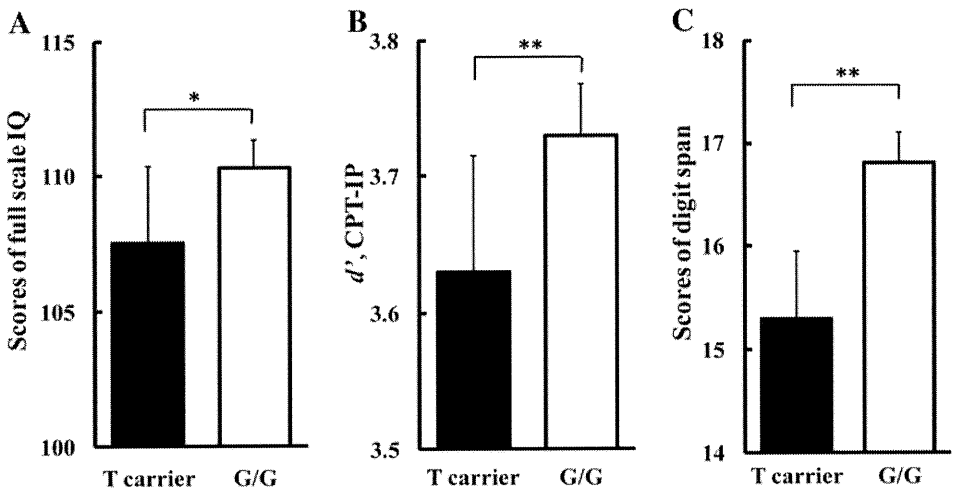

Figure 1. Association between the *KCNH2* risk genotype and IQ, attention/vigilance and working memory. IQ (A), attention/vigilance (B) and working memory (C). The *x*-axis represents T carriers and individuals with the G/G genotype. The *y*-axis represents scores of each test. Error bars represent the standard error of the mean. $*P < 0.05$, $**P < 0.01$.

Table II. Demographics of the combined studies.

	Authors	Ethnicities	Patients	Controls	Diagnostic criteria
	Case–control studies				
1	Huffaker et al. (2009)	German	905	1323	DSM-IV and ICD-10
2	Huffaker et al. (2009)	Armenian	161	161	ICD-10
3	Huffaker et al. (2009)	Italian	92	220	DSM-IV
4	Atalar et al. (2010)	Turkish	84	74	DSM-IV
5	Hashimoto et al. (present study)	Japanese	478	640	DSM-IV
	Family-based studies				
6	Huffaker et al. (2009)	USA (CBDB)	296 Caucasian families		DSM-IV
7	Huffaker et al. (2009)	USA (NIMH-GI)	71 Caucasian families		DSM-IIIR

CBDB, Clinical Brain Disorders Branch; NIMHGI, National Institute of Mental Health-Genetics Initiative.
We selected five independent case–control and two family-based data sets from previous and present studies. Two family-based samples (studies 6 and 7) were excluded from the present study because the published genotype data (affecteds and unaffecteds) were not available and the family-based samples with a family history of schizophrenia were not representative of the general population. Because we simply examined the association in the case–control samples, we included four independent case–control samples (studies 1, 2, 3, 4 and 5) (1,720 cases; 2,418 controls).

of the difference in allele frequency between patients and controls is consistent with previous studies (Huffaker et al. 2009; Atalar et al. 2010); however, the results did not represent a statistically significant difference between the groups ($z = 0.99$, $P = 0.32$, OR (95% confidence interval) = 1.15 (0.87–1.51)]. Our study size of 478 cases and 640 controls in a Japanese population had insufficient power (< 0.80) to detect as small an effect as an OR of 1.12, as described in the previous genome-wide association study (O'Donovan et al. 2008). Thus, we performed a meta-analysis to provide enough power to detect such a small effect. We included five independent case-control samples, as described in Table II (1,720 cases, 2,418 controls) (Huffaker et al. 2009; Atalar et al. 2010). The meta-analysis of M30 in all available schizophrenia data sets provided evidence for an association with schizophrenia ($z = 3.14$, $P = 0.0017$, OR (95% confidence interval) = 1.18 (1.06–1.31)] and no evidence for heterogeneity across studies ($Q = 3.55$, $P = 0.47$) (Table III, Figure 2). A sensitivity analysis revealed that the evidence for the association was not dependent upon the inclusion of any one data set (Supplementary Figure 1 available online).

Discussion

In this study, we replicated the association between the risk genotype *KCNH2* and IQ, and we further demonstrated the associations of the genotype with attention/vigilance and working memory in healthy Japanese subjects. We provided evidence that subjects with the risk T carriers had lower performances on these cognitive tests than did those with the G/G genotype. The effect sizes of the differences in these tests between individuals with T carriers and those with the G/G genotype were small to medium. Huffaker et al. reported a significant association between the M30 genotype and performance on IQ testing and on processing speed, which was extracted as a factor in healthy subjects (Huffaker et al. 2009). We did not find an association between processing speed and the risk genotype; however, we found associations between attention/vigilance and working memory and the risk genotype. To assess genotype

Table III. Comparison of allele frequencies of the *KCNH2* polymorphism (M30) in the combined samples.

	SCZ, Number of alleles (%)			CON, Number of alleles (%)			Statistics for each study		
M30 (rs3800779)	*T*	*G*	Sum	*T*	*G*	Sum	*P* value (*z*)	OR (95% CI)	Weight (fixed)
German	615 (34.0)	1195 (66.0)	1810	820 (31.0)	1826 (69.0)	2646	**0.035 (2.10)**	1.15 (1.01–1.30)	65.4
Armenian	105 (32.5)	217 (67.5)	322	87 (27.0)	235 (73.0)	322	0.13 (1.51)	1.30 (0.93–1.82)	9.2
Italian	51 (27.9)	133 (72.1)	184	114 (26.0)	326 (74.0)	440	0.63 (0.48)	1.10 (0.75–1.62)	7.1
Turkish	55 (32.7)	113 (67.3)	168	31 (20.9)	117 (79.1)	148	**0.020 (2.33)**	1.84 (1.10–3.06)	4.1
Japanese	105 (11.0)	851 (89.0)	956	124 (9.7)	1156 (90.3)	1280	0.32 (0.99)	1.15 (0.87–1.51)	14.1
Pool	931 (27.1)	2509 (72.9)	3440	1176 (24.3)	3660 (75.7)	4836	**0.0017 (3.14)**[a]	1.18 (1.06–1.31)	

SCZ, patients with schizophrenia; CON, healthy controls.
[a]Test of heterogeneity: $Q = 3.55$, df(Q) = 4, $P(Q) = 0.47$, $I^2 = 0$.

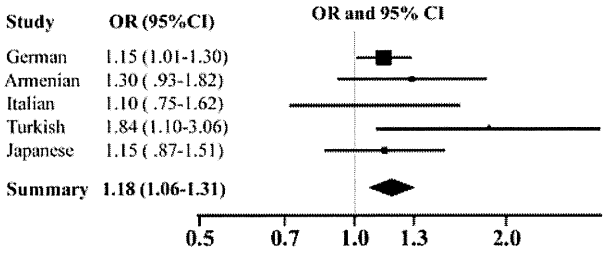

Figure 2. Forest plot of M30 results in the *KCNH2* gene based upon all combined populations. Solid squares and horizontal lines indicate the weighted odds ratios and 95% confidence intervals. The overall results are shown by the diamond. The results of the meta-analysis shown here are under the fixed-effects model.

effects on cognitive function, we measured seven domains based on MATRICS, a method different from the factor analyses-derived cognitive dimensions used in the previous study. Thus, the discrepancy between studies might be due to the differences in the cognitive dimensions, the methodology of the cognitive data analysis (direct measurements versus factors calculated by several measurements) and/or ethnic difference between European and Japanese individuals.

Second, we provide further evidence for an association between M30 and schizophrenia in combined case-control samples having now added a Japanese population. T allele frequencies of M30 in Japanese and European were different (~0.11 vs. ~0.27) based on previous and present genome dataset. However, there was no evidence for heterogeneity across studies in the meta-analysis, suggesting that there was no obvious population stratification in the combined case–control samples. It is important to note that our meta-analysis did not include the family-based sample that showed strong evidence for association in the original report. A leave-one-out sensitivity analysis revealed that the significant meta-analysis results were not being driven by a single data set. Removal of any one data set did not negate the significance of the association from the meta-analysis. As expected, the effect size observed in this study was quite small (1.16), consistent with the results from a GWAS report (O'Donovan et al. 2008). Our data are consistent with the concept that many susceptibility risk alleles for schizophrenia come from common variants of small effect. Our data also suggest that a common allele could have a stronger influence on intermediate phenotypes than on the diagnosis of schizophrenia. Despite the importance of cognitive deficits in schizophrenia, no drug has been approved for the treatment of this aspect of schizophrenia. Some antipsychotics bind and inhibit KCNH2 with affinities comparable to their affinities for the dopamine D2 receptors (Kongsamut et al. 2002). Further research will be required to clarify the role of *KCNH2* in the pathophysiology of schizophrenia. This research might potentially lead to new targets for antipsychotic medications.

There were several limitations to this study. We examined only M30 in the *KCNH2* gene, based on evidence that the variant predicts cognition, brain structure and function, and the gene expression level. We did not examine other markers of *KCNH2* gene or other genes to identify the association between those phenotype and schizophrenia. The lack of such association makes it unclear whether our results are directly linked with M30 or with other polymorphisms in linkage disequilibrium with this genetic variant. In addition, the neurocognitive tests batteries used in this study measure several complex functions (such as executive functions), not only associated with the one gene. Large number of researches show significant importance of genes connected with dopaminergic neurotransmitter system and other genes may interact with dopaminergic system (Tan et al. 2008b). Further study to investigate not only the single marker M30 but also these SNP/gene interactions is required.

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Statement of Interest

None to declare.

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