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Comprehensive association analysis of 27 genes from the GABAergic system in Japanese individuals affected with schizophrenia

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ABSTRACT

Involvement of the gamma-aminobutyric acid (GABA)-ergic system in schizophrenia pathogenesis through disrupted neurodevelopment has been highlighted in numerous studies. However, the function of common genetic variants of this system in determining schizophrenia risk is unknown. We therefore tested the association of 375 tagged SNPs in genes derived from the GABAergic system, such as $GABA_A$ receptor subunit genes, and GABA related genes (glutamate decarboxylase genes, GABAergic-marker gene, genes involved in GABA receptor trafficking and scaffolding) in Japanese schizophrenia case-control samples ($n = 2926$; 1415 cases and 1511 controls). We observed nominal association of SNPs in nine $GABA_A$ receptor subunit genes and the *GPHN* gene with schizophrenia, although none survived correction for study-wide multiple testing. Two SNPs located in the *GABRA1* gene, rs4263535 ($P_{allele} = 0.002$; uncorrected) and rs1157122 ($P_{allele} = 0.006$; uncorrected) showed top hits, followed by rs723432 ($P_{allele} = 0.007$; uncorrected) in the *GPHN* gene. All three were significantly associated with schizophrenia and survived gene-wide multiple testing. Haplotypes containing associated variants in *GABRA1* but not *GPHN* were significantly associated with schizophrenia. To conclude, we provided substantiating genetic evidence for the involvement of the GABAergic system in schizophrenia susceptibility. These results warrant further investigations to replicate the association of *GABRA1* and *GPHN* with schizophrenia and to discern the precise mechanisms of disease pathophysiology.

1. Introduction

Schizophrenia is a complex psychiatric disorder, manifesting heterogeneous behavioral and cognitive deficits, and afflicting approximately one percent of the global population (Owen et al., 2016). Overwhelming evidence supports neurodevelopmental abnormalities caused by genetic and environmental factors, in schizophrenia pathogenesis (Hashimoto et al., 2008b; Lewis and Levitt, 2002; Schmidt and Mirnics, 2015). In addition to the progressive symptoms seen in individuals affected with schizophrenia, observations of reduced cortical thickness, enlarged ventricles, reductions in gray matter volume, whole-brain volume, white matter anisotropy and a decreased neurogenic: gliogenic competence ratio further underscore neurodevelopmental dysfunction in disease pathology (Bakhshi and Chance, 2015; Harrison, 1999; Lewis and Lieberman, 2000; Toyoshima et al., 2016). In keeping with the neurodevelopment hypothesis, changes in neurodevelopment precede the onset of disease, affecting normal maturation, which in turn influences neuroplastic processes during development (Bakhshi and Chance, 2015).

The gamma-aminobutyric acid (GABA)-ergic system plays a significant role in neurodevelopment, by regulating neural proliferation, migration, differentiation, neuronal connectivity and synaptic activity (Deidda et al., 2014). Gamma-aminobutyric acid is the major inhibitory neurotransmitter in adult brains, and is synthesized from glutamate, by glutamate decarboxylases (GAD65 and GAD67) (Soghomonian and Martin, 1998). Its inhibitory function is mediated through the GABAA receptor, a heteropentameric ligand-gated chloride channel (Jacob et al., 2008). In addition, several receptor associated proteins aid in trafficking, tethering and lateral movement of GABAA receptors on the

Abbreviations: GABA, Gamma-aminobutyric acid; DSM-IV, The Diagnostic and Statistical Manual of Mental Disorders–IV; SNP, Single Nucleotide Polymorphism; LD, Linkage Disequilibrium.

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neuronal surface, thereby regulating GABAergic activity (Luscher et al., 2011).

The disruption of GABAergic function has been implicated in psychiatric diseases, like schizophrenia, for a long time (Gonzalez-Burgos et al., 2011; Nakazawa et al., 2012). Post-mortem studies have consistently showed abnormalities in parvalbumin-positive GABAergic interneurons, as well as altered expression of GABA related genes in the prefrontal cortex of individuals suffering from schizophrenia (Fung et al., 2010; Hoftman et al., 2015; Lewis et al., 2012; Lewis et al., 2005). In addition, impaired working memory, a characteristic feature of schizophrenia, has been attributed to aberrant gamma oscillations stemming from abnormal GABAergic interneuron activity in the prefrontal cortex of individuals affected with schizophrenia (Haenschel et al., 2009). Furthermore, development and maturation of the GABAergic system is also thought to be a convergent point for genetic and environmental susceptibility factors in schizophrenia (Schmidt and Mirnics, 2015).

Although post-mortem and animal studies showed GABAergic deficits in schizophrenia, human genetics data is limited to candidate gene association analysis and hampered by discrepant results (Cherlyn et al., 2010). A recent study showed for the first time that copy number variations (CNVs) are enriched for genes involved in GABAergic neurotransmission in schizophrenia cases (Pocklington et al., 2015). Moreover, results from our previous genome-wide association study (GWAS) in Japanese individuals affected with schizophrenia have shown association signals on the $GABA_A$ receptor subunit gene cluster at chromosome 5q34 (Yamada et al., 2011). In this study, we aimed to systematically investigate the role of common genetic variants from the GABAergic system in determining predisposition to schizophrenia. To this end, we performed a comprehensive case-control genetic association study of $GABA_A$ receptor subunit genes, and GABA related genes (glutamate decarboxylase genes, GABAergic-marker gene, genes involved in GABA receptor trafficking and scaffolding) in a large cohort of Japanese individuals affected with schizophrenia.

2. Methods

2.1. Subjects

The study examined 2926 unrelated Japanese case-control samples, consisting of 1415 individuals with schizophrenia (771 males, 644 females, mean age ± SD = 51.19 ± 13.68 years) and 1511 unrelated healthy controls (514 males, 997 females, mean age ± SD = 44.11 ± 14.04 years). The cohort also included samples from our previous GWAS study (120 probands from Japanese schizophrenia trio samples) (Yamada et al., 2011). Diagnosis of schizophrenia was based on Diagnosis and Statistical Manual of Mental Disorders IV (DSM-IV) criteria and confirmed by at least two experienced psychiatrists. Controls were interviewed by experienced psychiatrists, to exclude any past or present psychiatric disorders.

All the subjects were recruited from the Honshu area of Japan (the main island of Japan), where the population fall into a single genetic cluster (Yamaguchi-Kabata et al., 2008). Using a subset of subjects we previously showed that population stratification is negligible in our samples (Hattori et al., 2009; Yamada et al., 2011). All participants gave informed, written consent to join the study, after receiving a full explanation of study protocols and objectives. The study was approved by the ethics committees of RIKEN and all participating institutes, and was conducted in accordance with the Declaration of Helsinki.

2.2. Tag single nucleotide polymorphism (SNP) selection and genotyping

Genomic DNA was extracted from whole blood according to standard protocols. We selected pivotal genes in the GABAergic system for genetic analysis, such as $GABA_A$ receptor subunit genes, associated proteins involved in receptor stability, trafficking and scaffolding, and glutamate decarboxylase genes. Since deficits in GABAergic parvalbumin-positive interneurons are linked to schizophrenia pathogenesis, we also selected the GABAergic interneuron marker, parvalbumin for genetic analysis. Altogether, 19 $GABA_A$ receptor and 8 GABA related

Table 1
Information of genes selected for analysis.

Gene class	Gene name	Gene symbol	Chromosome	Number of SNPs selected for genotyping	Number of successfully genotyped SNPs
GABAA receptors	Gamma-aminobutyric acid (GABA) A receptor, alpha 1	*GABRA1*	5	8	8
	Gamma-aminobutyric acid (GABA) A receptor, alpha 2	*GABRA2*	4	9	7
	Gamma-aminobutyric acid (GABA) A receptor, alpha 3	*GABRA3*	X	22	21
	Gamma-aminobutyric acid (GABA) A receptor, alpha 4	*GABRA4*	4	16	15
	Gamma-aminobutyric acid (GABA) A receptor, alpha 5	*GABRA5*	15	12	10
	Gamma-aminobutyric acid (GABA) A receptor, alpha 6	*GABRA6*	5	5	5
	Gamma-aminobutyric acid (GABA) A receptor, beta 1	*GABRB1*	4	30	29
	Gamma-aminobutyric acid (GABA) A receptor, beta 2	*GABRB2*	5	29	28
	Gamma-aminobutyric acid (GABA) A receptor, beta 3	*GABRB3*	15	35	28
	Gamma-aminobutyric acid (GABA) A receptor, delta	*GABRD*	1	2	2
	Gamma-aminobutyric acid type A receptor epsilon subunit	*GABRE*	X	8	5
	Gamma-aminobutyric acid (GABA) A receptor, gamma 1	*GABRG1*	4	3	3
	Gamma-aminobutyric acid (GABA) A receptor, gamma 2	*GABRG2*	5	14	13
	Gamma-aminobutyric acid (GABA) A receptor, gamma 3	*GABRG3*	15	105	100
	Gamma-aminobutyric acid (GABA) A receptor, pi	*GABRP*	5	6	5
	Gamma-aminobutyric acid type A receptor theta subunit	*GABRQ*	X	4	3
	Gamma-aminobutyric acid (GABA) A receptor, rho 1	*GABRR1*	6	7	6
	Gamma-aminobutyric acid (GABA) A receptor, rho 2	*GABRR2*	6	17	16
	Gamma-aminobutyric acid (GABA) A receptor, rho 3	*GABRR3*	3	11	10
GABAA receptor associated proteins	GABA(A) receptor-associated protein	*GABARAP*	17	3	3
	Dystroglycan 1 (dystrophin-associated glycoprotein 1)	*DAG1*	3	2	2
	Dystrobrevin, beta	*DTNB*	2	20	20
	Gephyrin	*GPHN*	14	3	3
	N-ethylmaleimide-sensitive factor	*NSF*	17	2	1
Glutamate decarboxylase	Glutamate decarboxylase 1 (brain, 67 kDa)	*GAD1*	2	12	12
	Glutamate decarboxylase 2 (pancreatic islets and brain, 65 kDa)	*GAD2*	10	10	10
GABAergic neuron marker	Parvalbumin	*PVALB*	22	11	10
Total number of SNPs				406	375

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