GABRB2 Association with Schizophrenia: Commonalities and Differences Between Ethnic Groups and Clinical Subtypes

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Background: Single nucleotide polymorphisms (SNPs) and haplotypes in intron 8 of type A γ-aminobutyric acid ($GABA_A$) receptor β_2 subunit gene (GABRB2) were initially found to be associated with schizophrenia in Chinese. This finding was subjected to cross-validation in this study with Japanese (JP) and German Caucasian (GE) subjects.

Methods: Single nucleotide polymorphisms discovery and genotyping were carried out through resequencing of a 1839 base pair (bp) region in GABRB2. Tagging SNPs (tSNPs) were selected based on linkage disequilibrium (LD), combinations of which were analyzed with Bonferroni correction and permutation for disease association. Random resampling was applied to generate size- and gender-balanced cases and control subjects.

Results: Out of the 17 SNPs (9.2/kilobase [kb]) revealed, 6 were population-specific. Population variations in LD were observable, and at least two low LD points were identified in both populations. Although disease association at single SNP level was only shown in GE, strong association was demonstrated in both JP ($p = .0002 – .0191$) and GE ($p = .0033 – .0410$) subjects, centering on haplotypes containing rs1816072 and rs1816071. Among different clinical subtypes, the most significant association was exhibited by systematic schizophrenia.

Conclusions: Cross-population validation of GABRB2 association with schizophrenia has been obtained with JP and GE subjects, with the genotype-disease correlations being strongest in systematic schizophrenia, the most severe subtype of the disease.

Key Words: Complex disease, haplotype, population association, psychiatric disorder, SNP

Schizophrenia is a debilitating brain disease, characterized by impaired cognitive and affective processing (Austin 2005; Elvevag and Goldberg 2000; Shayegan and Stahl 2005) and affecting approximately 1% of the world population (Gottesman 1989). Twin and adoption studies have furnished evidence for a substantial genetic contribution to schizophrenia susceptibility (Cardno and Gottesman 2000; Ingraham and Kety 2000). Since pathology in the brain of schizophrenia patients (Clinton and Meador-Woodruff 2004; Harrison 2004; Harrison and Weinberger 2005) and antipsychotic or psychotic-mimetic effects of some chemical compounds (Lyne et al 2004) such as phencyclidine (Morris et al 2005) both point to disturbances in neurotransmitter systems in schizophrenia, it is plausible that disease-related polymorphisms exist in the genes of neurotransmission pathways (Kennedy et al 2003; Morris et al 2005; Owen et al 2004).

Gamma-aminobutyric acid (GABA)ergic neurons contribute to the orchestration of pyramidal neuron firing, and GABAergic cortical deficits are implicated in neurodevelopmental abnormality and schizophrenia pathophysiology (Caruncho et al 2004; Costa et al 2004; Guidotti et al 2005; Heckers and Konradi 2002; Yee et al 2005). Following the initial proposal of GABAergic defects in schizophrenic patients (Roberts 1972), potential disturbances of the GABA pathway in schizophrenia have been scrutinized (Korpi and Sinkkonen 2006). Muscimol binding to type A γ-aminobutyric acid ($GABA_A$) receptors is altered in schizophrenic patients in the anterior cingulate cortex (Benes et al 1992), the hippocampal formation (Benes et al 1996a), and the prefrontal cortex (Benes et al 1996b). Other observable changes pertain to the expression of $GABA_A$ receptor α_1 and $\beta_{2/3}$ subunits in the prefrontal cortex (Ishikawa et al 2004) and measurements of prepulse inhibition (Braff et al 2001, 2005; Hauser et al 2005). A group of $GABA_A$ receptor genes for β_2, α_6, α_1, γ_2, and π subunits on chromosome 5q is in the neighborhood of schizophrenia candidate loci suggested by linkage studies (Gurling et al 2001; Moises et al 2002; Schwab et al 1997; Straub et al 1997).

Positive association between schizophrenia and single nucleotide polymorphism (SNP) haplotypes in the $GABA_A$ receptor β_2 subunit gene (*GABRB2*) was observed earlier in a Chinese population-based study (Lo et al 2004). This observation was later confirmed by two independent family-based studies, one with a set of Chinese samples (Liu et al 2005) different from that used by Lo et al (2004) and the other with German and Portuguese samples (Petryshen et al 2005). A study of Japanese population (Ikeda et al 2005) did not indicate any schizophrenia association, even though two of the eight tagging SNPs in *GABRB2* genotyped, namely rs6556547 and rs187269, overlapped with those analyzed by Lo et al (2004).

Accordingly, the present study aimed to cross-check the findings by Lo et al (2004) in two geographically divergent Japanese (JP) and German Caucasian (GE) ethnic populations. In addition, potential genetic heterogeneity among different clinical subtypes of schizophrenia was examined based on diagnostic information available for the GE samples. In view of potential sequence variations among different populations, extensive re-

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sequencing rather than mere genotyping of a few discrete markers was performed for the *GABRB2* region harboring the SNPs associated with Chinese schizophrenic patients to not miss any population-specific SNPs. Based on the linkage disequilibrium (LD) patterns, tagging SNPs (tSNPs) were selected for the JP and GE samples. Disease association analysis was exhaustively performed for all the individual SNPs, as well as all combinations of tSNPs. Both Bonferroni correction and permutation test were applied to the significant associations. To minimize the effects of unequal sample size and gender ratio between the disease and control groups, extensive resampling was also performed.

Methods and Materials

Subjects

A total of 511 JP and 491 GE samples were analyzed. The JP samples consisted of 207 unrelated healthy individuals (female subjects 105; male subjects 102; mean age 48.8 ± 23.9 years) and 304 unrelated schizophrenia patients (female patients 94; male patients 210; mean age 54.8 ± 13.3 years). The GE samples consisted of 190 unrelated healthy control subjects (female subjects 76; male subjects 114; mean age 29.3 ± 9.0 years) and 301 unrelated schizophrenia patients (female patients 59; male patients 242; mean age 28.3 ± 10.1 years). The GE patient samples were collected from subjects displaying two clinical subtypes according to the classification of endogenous psychoses of Leonhard (1999): unsystematic schizophrenia (female subjects 32; male subjects 123; mean age of onset 32.1 ± 11.7 years) and systematic schizophrenia (female subjects 27; male subjects 119; mean age of onset 24.3 ± 8.3 years). There was no overlap in samples used in this study and those used by Yu et al (2006). Interrater reliabilities on diagnosis and collections of GE samples were as described (Pfuhlmann et al 1997; Stöber et al 2002).

All of the GE and JP schizophrenia patients were hospitalized in the hospitals of the Department of Psychiatry and Psychotherapy, University of Wuerzburg, Germany, and Department of Neuropsychiatry, Kurume University School of Medicine, Fukuoka, Japan, respectively, and fulfilled the diagnostic criteria for schizophrenia in DSM-IV (American Psychiatric Association 1994). Control groups for both populations were free from any present, past, and family history of psychiatric illness or substance abuse diagnosis. Each patient or control subject was accessed by at least two experienced psychiatrists. Following presentation of a complete plan of the present study, written informed consent was obtained from each subject prior to study. Approval for the study was obtained from the Ethics Committees of Kurume University for the JP samples and of University of Wuerzburg for the GE samples.

DNA Samples and Polymerase Chain Reaction

Genomic DNA was isolated from peripheral blood of subjects, and a region of 1839 base pair (bp) spanning from SNP rs252944 to SNP rs6556547, located 2041 bp to 203 bp upstream of exon 9 of *GABRB2* (Contig accession number: NT_023133.12; messenger RNA [mRNA] accession number: NM_021911), was amplified by polymerase chain reaction (PCR) and sequenced as described (Lo et al 2004). Primers were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) (Rozen and Skaletsky 2000). Specificity of primers was validated against the human genome database of the National Center for Biotechnology Information with BLASTN (http://www.ncbi.nlm.nih.gov/BLAST/). The forward and reverse primers designed to amplify a 2801 bp PCR product covering the region of interest were 5′-TGGAGGAAAGGTCCATATCTAGT-3′ and 5′-TTGTAAAGCTATTGTCCAGCAAGT-3′. Both of them yielded no more than five matches to the rest of the human genome with the default parameters: Low Complexity Filter on, Expect Value of 10, and Word Size of 11.

Amplification of DNA fragment was performed in a 20 μL PCR mixture containing 10 ng of genomic DNA, 75 nmol/L of each primer, 50 nmol/L of each deoxyribonucleotide triphosphate (dNTP), 2.5 mmol/L magnesium chloride ($MgCl_2$), and 1 U *Taq* DNA polymerase (Amersham Bioscience, Uppsala, Sweden). Polymerase chain reaction consisted of initial denaturation at 94°C for 5 minutes, followed by 35 cycles each of 30 seconds at 95°C, 30 seconds at 60°C, 90 seconds at 72°C, plus a final extension step at 72°C for 5 minutes. The PCR product in each instance was resolved on 1.0% agarose gels and stained with ethidium bromide to confirm the presence of PCR product with the expected size and the absence of nonspecific products. To purify the PCR product, absolute ethanol of analytical grade was added to the post-PCR reaction mixture to a final concentration of 75%, and the mixture was kept at 4°C for at least 1 hour. After centrifugation at 3000 rpm for 30 minutes at room temperature, the precipitates were washed three times with 70% ethanol with centrifugation between washes at 4000 rpm for 15 minutes. The air-dried PCR product precipitates were dissolved in 1 × Tris-Cl (USB, Cleveland, Ohio) and ethylenediaminetetraacetic acid (EDTA, Grand Island, New York) buffer for subsequent sequencing.

SNP Discovery and Genotyping

Both SNP discovery and genotyping were obtained by direct sequencing of the PCR products. Four sequencing primers were empolyed: 5′-AAACACTATCCAATAACGCATCCT-3′, 5′-CCTCTAAGCTGTAATCGGAAGGTA-3′, 5′-CCTAATGGGGGAGTTTGAAC-3′, and 5′-CTTAATAGCTGGAAAGGTGAT-3′. Each sequencing reaction contained .5 μL of BigBye Terminator version 3.1 (Applied Biosystems Inc., Foster City, California), ~100 ng purified PCR products, and 1 mmol/L sequencing primer. Each cycle of sequencing reaction consisted of initial denaturation at 96°C for 1 minute, followed by 25 cycles each of 10 seconds at 96°C, 5 seconds at 50°C, and 4 minutes at 60°C. Ethanol precipitation was employed to clean up the postsequencing reactions as described for the PCR products. Each air-dried sequencing sample was dissolved in 10 μL Hi-Deionized Formamide (Applied Biosystems Inc.), denatured at 95°C for 1 minute and immediately kept at 4°C prior to sequencing with a Model 3100 Genetic Analyzer (Applied Biosystems Inc.).

Sequence chromatogram alignment-based SNP discovery and genotype calling were carried out using the software package PolyPhred version 4.2 (http://draog.mbt.washington.edu/polyphred.html) (Nickerson et al 1997). All genotyping results were manually confirmed by at least two independent researchers. All analyzed SNPs were located within the high-quality region (Quality Value ≥ 20), and occasional low-quality passes were resequenced.

Hardy-Weinberg Equilibrium

Exact test of Hardy-Weinberg equilibrium (HWE) was performed for the healthy control samples from the two ethnic populations by the Markov chain method with the programs Arlequin version 2.0 (http://lgb.unige.ch/arlequin/) (Schneider et al 2000) and GENEPOP version 3.4 (http://wbiomed.curtin.edu.au/genepop/). The following parameters were employed for both programs: forecasted chain length = 100,000 (100 batches