Table 1
Genotype and allele distributions for the H204R polymorphism of the chimerin 2 gene between the patients with schizophrenia and controls

Group	N	Genotype distribution				Mantel Haenszel	Allele frequency			odds ratio (95%CI)
		His/His	His/Arg	Arg/Arg	P value	P value	His	Arg	P value	
Male										
Patients	162	95 (58.6%)	57 (35.2%)	10 (6.2%)	0.047	0.018	247 (76.2%)	77 (23.8%)	0.018	1.53 (1.07–2.19)
Controls	222	152 (68.4%)	65 (29.3%)	5 (2.3%)			369 (83.1%)	75 (16.9%)		
Female										
Patients	131	80 (61.2%)	44 (33.6%)	7 (5.3%)	0.703	0.678	204 (77.9%)	58 (22.1%)	0.681	1.08 (0.75–1.56)
Controls	228	141 (61.9%)	79 (34.6%)	8 (3.5%)			361 (23.8%)	95 (20.8%)		
Total										
Patients	293	175 (59.7%)	101 (34.5%)	17 (5.8%)	0.087	0.052	451 (77.0%)	135 (23.0%)	0.053	1.29 (1.00–1.66)
Controls	450	293 (65.1%)	144 (32.0%)	13 (2.9%)			730 (81.1%)	170 (18.9%)		

more common in the cases than in the controls ($\chi^2=3.94$, $df=1$, $p=0.049$, odds ratio = 2.07, 95%CI 0.99–4.33). The observed frequency for the minor allele (R204) in our control group (19%) was quite similar to that reported by Haga et al. (2002) (18%) estimated from 48 Japanese chromosomes. Thus, the observed significant difference in the allele frequency between the cases and controls cannot be ascribed to an unusually lower frequency of the R204 allele in our control subjects.

As gender differences occur in various aspects of the disease, including earlier age of onset, poorer course and medication response in men, we examined males and females separately. The R204 allele was excess in our cases when compared to controls among males ($\chi^2=5.57$, $df=1$, $p=0.018$, odds ratio = 1.53, 95%CI 1.07–2.19). Genotype distributions also revealed significant difference between male controls and male patients with schizophrenia ($\chi^2=6.12$, $df=2$, $p=0.047$; $\chi^2=5.56$, $df=1$, $p=0.018$ by Mantel Haenszel test). However, there was significant difference in neither allele frequency nor genotype distribution between the schizophrenics and controls in females.

CHN2 protein acts as a receptor of diacylglycerol/phorbol esters and regulates the activity of the Rac GTPase, one of the Rho GTPase family proteins (Caloca et al., 2003). The CHN2 inhibits Rac-GTP activation by the stimulation of epidermal growth factor (EGF). EGF protein levels were decreased in the prefrontal cortex of schizophrenic patients, and conversely, EGF receptor expression was elevated in the prefrontal cortex (Futamura et al., 2002). Serum EGF levels were markedly reduced in schizophrenic patients, even in young, drug-free patients (Futamura et al., 2002). Neonatal perturbation of EGF in rats resulted in abnormal sensorimotor gating and social interaction in adults (Futamura et al., 2003). In addition, neuregulin-1, one of the EGF family proteins, was reported as a schizophrenia susceptibility gene (Harrison and Owen, 2003) and the abnormal expression of neuregulin-1 has been observed in schizophrenic brain (Hashimoto et al., 2003). Therefore, the CHN2 H204R polymorphism might lead to the abnormality of neuregulin signaling pathways. As the location of H204R is close to diacylglycerol/phorbol ester binding domain (214–264 amino acid), this polymorphism could alter the protein structure of the region, which may change the second messenger signaling. H204R polymorphism, next to a casein kinase II phosphorylation site, might also play a potential role in the CHN2 phosphorylation state, although the physiological phosphorylation status is unclear.

We demonstrated, for the first time, the possible association between a missense polymorphism (H204R) of the CHN2 gene and schizophrenia in a Japanese population. A false-positive association due to population stratification could not be excluded in our case control designed study, despite the precaution of ethnic matching of this study. Therefore, it is necessary to carry out further investigations to confirm our findings in other samples. If our results are replicated, functional analysis of the CHN2 H204R polymorphism might contribute to understanding the molecular mechanisms of schizophrenia.

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No association of haplotype-tagging SNPs in TRAR4 with schizophrenia in Japanese patients

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Abstract

Recent study of linkage disequilibrium mapping showed one of the trace amine receptor (TRAR) genes, TRAR4, was associated with schizophrenia. We conducted a replication study of TRAR4 with schizophrenia in Japanese patients. We used two large independent sets of samples in a first-set analysis (cases=405, controls=401) and second-set analysis (cases=503, controls=440). In the first-set analysis, one Marker (Marker5) showed a significant association, but this significance was not seen in the second-set analysis. Our results indicate that TRAR4 may not play a major role in Japanese schizophrenia patients, and that it is important to examine the possibility of false positives in genetic association analysis.

Keywords: Schizophrenia; Trace amine receptor; Linkage disequilibrium; Haplotype-tagging SNP

1. Introduction

Trace amines (TAs) are endogenous amine compounds that are chemically similar to classic biogenic amines. TAs were thought to be 'false transmitters' which displace classic biogenic amines from their storage and act on transporters in a fashion similar to the amphetamines, but the identification of brain receptors specific to TAs indicates that they also have effects of their own effects, and TA receptors bind several psychostimulants such as amphetamine and D-lysergic acid diethylamide (LSD) (Parker and Cubeddu, 1986).

A recent study of linkage disequilibrium (LD) mapping showed significant association between SNPs in one trace amine receptor (TRAR) gene TRAR4 and schizophrenia (SCZ) (Duan et al., 2004). By genotyping 192 pedigrees with SCZ of European or African American ancestry, from samples that previously showed linkage evidence to 6q13–

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q26, an association of schizophrenia with 1 SNP (rs4307545: Marker16) within the TRAR4 gene remained significant after correction for multiple testing. The authors also showed that TRAR4 is preferentially expressed in several brain regions including the hippocampus that have been implicated in the pathophysiology of SCZ.

In this study, we conducted a replication study of TRAR4 with SCZ in Japanese patients. However, because SNP variations and LD patterns differ among populations, we included a systematic mutation search around TRAR4 and an LD evaluation. After the selection of haplotype-tagging (ht) SNPs, we performed a two-stage association analysis using two independent sets of samples in a first-set analysis (cases=405, controls=401) and second-set analysis (cases=503, controls=440).

2. Materials and methods

2.1. Subjects

In the association analyses, two independent sets of samples were examined: for the first-set screening scan, 405 patients with SCZ (206 male and 199 female; mean age ± standard deviation (S.D.) 42.6 ± 14.9 years) and 401 controls (213 male and 188 female; 34.1 ± 13.1 years), and for the second-set confirmation analysis, 503 patients with SCZ (284 male and 219 female; 52.8 ± 13.8 years) and 440 controls (223 male and 217 female; 40.7 ± 14.2 years).

The subjects for mutation search and LD evaluation were 37 patients with SCZ and 64 controls, respectively. These subjects were also included in the first-set screening scan. Characterization details and psychiatric assessment of these subjects were identical to those published elsewhere (Ikeda et al., 2005). All subjects were unrelated to each other and ethnically Japanese.

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

2.2. Mutation search

Primer pairs were designed using information from the GenBank sequence (accession number: NT-025741.13) and 5 amplified regions, which covered the coding exon and 5′ flanking region upstream 500 bp. A more detailed description can be seen in a previous paper (Suzuki et al., 2003). Sequences of primer pairs are available on request.

2.3. SNP selection and LD evaluation

For the evaluation of LD, we included the positive SNPs shown in the original paper (Duan et al., 2004) (Fig. 1) in addition to the SNPs we detected. First we determined 'LD blocks' with criteria based on 95% confidential bounds on D' using HAPLOVIEW ver 3.0 software (Barrett et al., 2005). Next, htSNPs were selected within each LD block for 90% haplotype coverage using SNPtagger software (Ke and Cardon, 2003).

2.4. SNP genotyping

We used TaqMan assays (Marker5, 12, 16, 19 and 21), primer extension using denaturing high performance liquid chromatography (Marker3, 4, 6, and 14) and direct sequencing (MarkerA and B). Sequences of primer pairs are available on request.

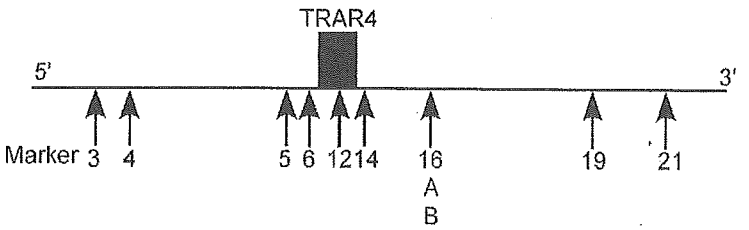

Fig. 1. Genomic structure of TRAR4. Numbers under arrows represent marker IDs. Vertical bar represents exon.

2.5. Statistical analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by χ^2 test (SAS/Genetics, release 8.2, SAS Institute Japan Inc, Tokyo, Japan).

Marker-trait association was evaluated allele/genotype-wise with χ^2 test (SPSS 10.0J, SPSS Japan Inc). In the first-set screening scan, we used a recently developed software program, SNPSpD, which could reflect the correlation of markers (LD) on corrected *P*-values, in order to control inflation of the type I error rate (Nyholt, 2004).

Power calculation was performed using a statistical program prepared by Ohashi et al. (2001). We estimated the power for our sample size under a multiplicative model of inheritance.

The significance level for all statistical tests was 0.05.

3. Results

Searched variants in the coding exon and 5′ flanking region among this ethnic group, two SNPs were identified in the 5′ upstream and 3′ downstream of TRAR4 (Marker6 and 14). None of the other SNPs were discovered.

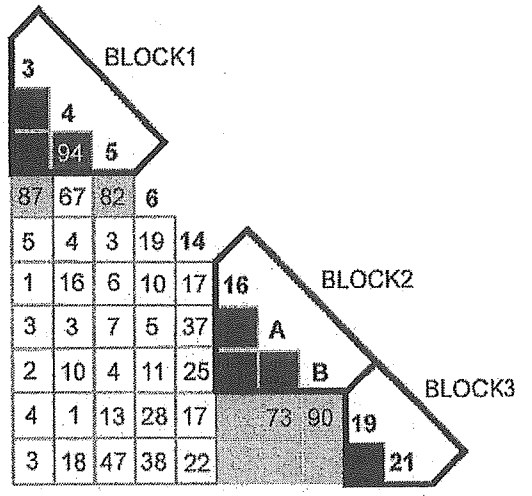

Fig. 2. Linkage disequilibrium evaluation. Numbers in box represents *D′* values after decimal point. *D′* values of 1.0 are not shown. Other information is provided at Haploview's website.

Nine SNPs of the Duan's report and two SNPs we detected were genotyped for evaluation of LD. All genotype frequencies of these SNPs were in HWE. Marker12 was excluded from the LD evaluation due to its low minor allele frequency (0.8%). Three LD blocks were defined and six SNPs (five htSNPs and Marker12) were selected (Table 1 and Fig. 2).

In the association analysis, we first genotyped these htSNPs and Marker12 for first-set samples (cases=405,

Table 1
htSNPs and first-set screening scan

Marker	SNP ID[a]	Blocks[b]	MAF[c] (%)	Genotype distribution[d]									
				M/M		M/m		m/m		*P*-value[e]		Corrected *P*-value[f]	
				SCZ	CON	SCZ	CON	SCZ	CON	Genotype	Allele	Genotype	Allele
3	rs4473885 (G > A)		18.0										
4	rs4085406 (A > G)	BLOCK I	19.5										
5	rs6907909 (A > G)		24.2	233	266	150	119	22	16	**0.0354**	**0.0122**	0.204	0.0702
6	rs9373026 (C > G)		48.4	143	132	179	181	83	88	0.749	0.437		
12	rs8192625 (A > G)		0.80	394	389	11	12	0	0	0.814	0.815		
14	rs7772821 (T > G)		28.1	243	231	143	145	19	25	0.572	0.350		
16	rs4305745 (G > A)		21.9	151	162	181	186	73	53	0.165	0.102		
A	rs17078770 (–/A)	BLOCK II	20.3										
B	rs7452939 (C > T)		21.1										
19	rs6903874 (T > C)		14.1										
21	rs6937506 (G > A)	BLOCK III	13.3	298	292	96	96	11	13	0.901	0.716		

[a]Gray box represents 'haplotype-tagging (ht) SNPs' for association analysis.
[b]Blocks were defined by HAPLOVIEW.
[c]MAF=minor allele frequency of 64 controls.
[d]M=major allele, m=minor allele, SCZ=schizoprenia, CON=control.
[e]Bold numbers represent significant *P*-values.
[f]Corrected *P*-values were calculated by SNPSpd.

Table 2
Second-set confirmation analysis of Marker5

	Phenotype[a]	Genotype[b]			Allele[c]	*P*-value	
		A/A	A/G	G/G	MAF (%)	Genotype	Allele
Marker5	SCZ	305	175	23	22.0	0.662	0.576
	CON	271	154	15	20.9		

[a] SCZ=schizoprenia, CON=controls.
[b] M=major allele, m=minor allele.
[c] MAF=minor allele frequency.

controls=401). Marker-trait association showed an association Marker5 to SCZ ($P=0.0354$; genotype, $P=0.0122$; allele) (Table 1). However, after correction for the type I error rate by using program SNPSpD, corrected *P*-value became 0.204 for genotype and 0.0702 for allele, respectively (Table 2: effective number of independent loci, 5.7719, experiment-wide significance threshold required to keep type I error rate at 0.05, 0.008662).

To confirm Marker5 association, we performed a second-set analysis of Marker5 using an independent panel of samples (cases=503, controls=440). In this analysis, there was no association SCZ to Marker5 (Table 2).

We included a power calculation, and obtained more than 80% power to detect association when we set the genotype relative risk at 1.36 under a multiplicative model of inheritance.

4. Discussion

Through two-stage association analysis, htSNPs in TRAR4 were not found to be associated with SCZ in Japanese patients. Our results indicate the great importance of examining the possibility of false positives in genetic association analysis. False positives may be produced by population stratification. However, this might not be the case with our results, which instead may have derived from inflation of the type I error rate due to multiple testing, since the Japanese population is believed to be quite homogeneous.

We also included a haplotypic analysis of Marker5 and 6, which were relatively strong LD, using first-set samples (SAS/Genetics). Again, we could find no significant association ($P=0.0734$). This result also supports the possibility of a false positive for Marker5.

The strategy adopted in this study was a powerful one owing to the method of htSNP selection and two-stage association analysis. Moreover, by performing a mutation search with enough power to detect rare polymorphisms, we could avoid overlooking associations in accordance with the common disease–rare variant hypothesis (Pritchard, 2001).

In conclusion, we could not replicate the association of TRAR4 and SCZ using a Japanese population. Further replication analysis using different population samples will be required for conclusive results.

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Association study of polymorphisms in the GluR7, KA1 and KA2 kainate receptor genes (*GRIK3*, *GRIK4*, *GRIK5*) with schizophrenia

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Abstract

On the basis of the glutamatergic dysfunction hypothesis of schizophrenia, we have been conducting a systematic study of the association of glutamate receptor genes with schizophrenia. Here we report association studies of schizophrenia with polymorphisms in three kainate receptor genes: *GRIK3*, *GRIK4* and *GRIK5*. We selected 16, 24 and 5 common single nucleotide polymorphisms (SNPs) distributed in the entire gene regions of *GRIK3* (>240 kb), *GRIK4* (>430 kb) and *GRIK5* (>90 kb), respectively. We tested associations of the polymorphisms with schizophrenia using 100 Japanese case–control pairs (the Kyushu set). We observed no significant "single marker" associations with the disease in any of the 45 SNPs tested except for one (rs3767092) in *GRIK3* showing a nominal level of significance. The significant association, however, disappeared after the application of the Bonferroni correction. We also observed significant haplotype associations in seven SNP pairs in *GRIK3* and in four SNP pairs in *GRIK4*. None, however, remained significant after Bonferroni correction. We also failed to replicate the nominally significant haplotype associations in a second sample set, the Aichi set (106 cases and 100 controls). We conclude that SNPs in the gene regions of *GRIK3*, *GRIK4* or *GRIK5* do not play a major role in schizophrenia pathogenesis in the Japanese population.

Keywords: Schizophrenia; Association study; Glutamate receptor; Kainate receptor gene; SNP; Linkage disequilibrium; Haplotype analysis

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1. Introduction

Reports of psychotic symptoms induced by phencyclidine (PCP) have stimulated interest in the possible role of a glutamatergic dysfunction in the pathogenesis of schizophrenia (Snyder, 1980; Javitt and Zukin, 1991). We have been conducting a systematic study of associations of the glutamate receptor (GluR) gene family with schizophrenia (Joo et al., 2001; Shibata et al., 2001, 2002; Tani et al., 2002; Makino et al., 2003; Fujii et al., 2003; Takaki et al., 2004). We have selected a set of common single nucleotide polymorphisms (SNPs) covering the entire genomic region of the target gene. By examining their allele frequencies and linkage disequilibria (LD), we tested individual associations and haplotype associations of the SNPs with the disorder. We earlier reported the absence of a significant association of schizophrenia with two genes encoding low-affinity kainate receptor subunits, GluR5 and GluR6 (Shibata et al., 2001, 2002). Here we report association studies of genes encoding the remaining three members of the kainate receptor subfamily, i.e., GluR7, KA1 and KA2, with schizophrenia.

The GluR7 receptor gene *GRIK3* is located on chromosome 1p34-33, where a significant linkage with schizophrenia has been reported (DeLisi et al., 2002). Significant changes of GluR7 expression in schizophrenia have been reported in multiple brain regions (Sokolov, 1998; Meador-Woodruff and Healy, 2000; Benes et al., 2001). The KA1 kainate receptor gene *GRIK4* is located on chromosome 11q22.3, where the fourth strongest linkage has been observed in a meta-analysis compiling 20 genome scans (Lewis et al., 2003). A significant decrease of KA1 mRNAs as well as of NMDAR1 and GluR1 mRNAs has been reported in the frontal cortex of neuroleptic-free patients with schizophrenia (Sokolov, 1998). The KA2 kainate receptor gene *GRIK5* is located on chromosome 19q13.2, where no linkage with schizophrenia has been previously reported. However, significant changes of KA2 expression in schizophrenia have been reported in multiple brain regions (Porter et al., 1997; Ibrahim et al., 2000; Meador-Woodruff and Healy, 2000). These lines of evidence suggest that *GRIK3*, *GRIK4* and *GRIK5* are strong candidates as susceptibility genes for schizophrenia.

In this report, we tested associations of schizophrenia with 16, 24 and 5 common SNPs selected from the entire regions of *GRIK3*, *GRIK4* and *GRIK5*, respectively. To enhance the detection power of haplotype association, the SNPs were placed depending on the magnitude of linkage disequilibrium (LD) in each subregion. That is, the weaker the observed LD, the more SNPs tested in the subregion. By testing haplotype associations in each subregion, we extensively examined associations of the entire gene regions with schizophrenia.

2. Methods

2.1. Subjects

Blood samples were obtained from unrelated Japanese individuals who provided written informed consent. We studied 100 schizophrenia patients recruited from hospitals in the Fukuoka and Oita areas (mean age=49.5; 44% female) and 100 healthy controls recruited from the Fukuoka area (mean age=51.2; 44% female) (the Kyushu set). To evaluate the significance of findings in the Kyushu set, we used another sample set, the Aichi set, which comprised 106 cases (mean age=39.4; 45.5% female) and 100 cases (mean age=34.4; 41.9% female) recruited from the Aichi area, about 600 km east of Fukuoka. All patients fulfilled the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for schizophrenia. This study was approved by the Ethics Committee of Kyushu University, Faculty of Medicine and by the Fujita Health University Ethics Committee. Genomic DNA was purified from leukocytes as previously described (Lahiri and Nurnberger, 1991).

2.2. SNP selection in the GRIK3, GRIK4 and GRIK5 regions

We retrieved all the primary information on SNPs from the public database, dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) and a private database, Celera Discovery System (http://www.celeradiscoverysystem.com). We examined their allele frequencies in 16 healthy Japanese samples by the direct sequencing method as previously described (Shibata et al., 2002). When a tested SNP showed an insufficient

polymorphism (<10%), we selected another SNP close by the rare SNP and examined its allele frequency in the same way. Also we analyzed LD between the neighboring SNPs as described below. As we intended to cover the entire region with moderate-to-strong LD for haplotype association tests, we selected additional SNPs in the subregions showing very weak LD (D' <0.3). For a direct test of functional variants, we also examined the frequency of all exonic SNPs available on the database and included them in the association analyses if they were common (>10%).

2.2.1. GRIK3

After the examination of 182 SNPs from the database, we selected the following 16 common SNPs distributed in the *GRIK3* region spanning over 240 kb: SNP1, rs551794; SNP 2, rs534131; SNP3, rs822856; SNP4, rs3767100; SNP5, rs3753771; SNP6, rs3767092; SNP7, rs3767086; SNP8, rs554445; SNP9, rs1160751; SNP10, rs1334804; SNP11, rs3767067; SNP12, rs550250; SNP13, rs565537; SNP14, rs3767048; SNP15, rs3767045; SNP16, rs2993076. All SNPs are located in noncoding regions except for one synonymous SNP, SNP12 in exon 3 (Fig. 1a).

2.2.2. GRIK4

Since we found a discrepancy at the 5′ ends in two GenBank entries, XM_166179 and NM_014619 for the *GRIK4* cDNA sequences, we selected both exons 1A and 1B as potential alternative transcription initiation sites. After the examination of 87 SNPs from the database, we selected the following 24 common SNPs distributed in the *GRIK4* region spanning over 430 kb: SNP1, rs2248404; SNP 2, rs1317176; SNP3, rs1317514; SNP4, rs1343789; SNP5, rs1893906; SNP6, rs2000870; SNP7, rs2000868; SNP8, rs1939664; SNP9, rs2852227; SNP10, rs3133226; SNP11, rs3133845; SNP12, rs2852230; SNP13, rs2846092; SNP14, rs2846103; SNP15, rs4359220; SNP16, rs4936552; SNP17, rs2004676; SNP18, rs3824978; SNP19, rs3802911; SNP20, rs2156637; SNP21, rs2156635; SNP22, rs2156634; SNP23, rs2298725; SNP24, rs611065. SNP19, SNP22 and

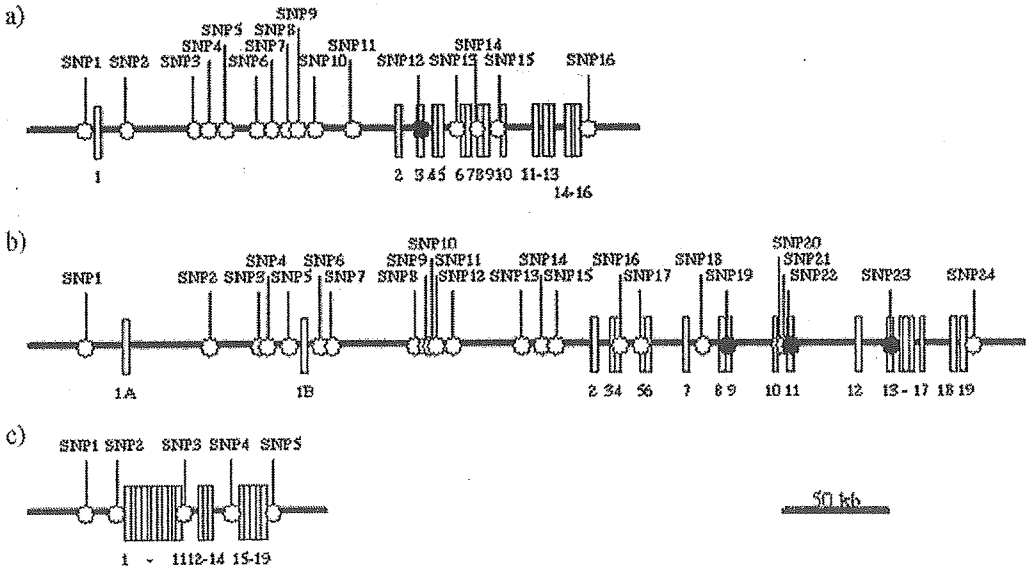

Fig. 1. Genomic organizations of *GRIK3*, *GRIK4* and *GRIK5*, and locations of the SNPs. (a) *GRIK3* spans over 233 kb and is composed of 16 exons shown as boxes with exon numbers. Circles indicate the 16 common SNPs we analyzed. (b) *GRIK4* spans over 410 kb and is composed of 19 exons shown as boxes with exon numbers. Circles indicate the 24 common SNPs we analyzed. (c) *GRIK5* spans over 67 kb and is composed of 19 exons shown as boxes with exon numbers. Circles indicate the 5 common SNPs we analyzed. Four exonic SNPs (one in *GRIK3* and three in *GRIK4*) are indicated by filled circles.

Table 1
PCR primers for genotyping of SNPs in *GRIK3, 4, 5*

Gene	SNP	Primer sequence (5′-3′)	Product size (bp)	Annealing temp (°C)	Cycle number	Typing method[a]
GRIK3	SNP1	Forward GGAAGGGAGCTGAAGGTAGG	211	63	35	RFLP
	rs551794	Reverse CCTGTGCACGTGTTTTGTTT				(*Bsp* HI)
	SNP2	Forward GGCTGTGTGAGGGCAGAC	354	60	30	RFLP
	rs534131	Reverse CCCGATTCTACTGGGACCTT				(*Pst* I)
	SNP3	Forward CCAGTGCCCAGAATCATATT	248	60	40	RFLP
	rs822856	Reverse GAAGGGCTGCAGTGAATGAC				(*Pvu* II)
	SNP4	Forward TCACCCTCGATGTAACCTTC	281	60	35	DS
	rs3767100	Reverse TTGCTTTCTAGCCGAGTCCA				
	SNP5	Forward AGGGAAGACACGGAACTCAC	330	60	35	DS
	rs3753771	Reverse ACACCTGGCATCAAGCATC				
	SNP6	Forward CCTCTTCCCCTCTCTTGCTT	291	60	40	RFLP
	rs3767092	Reverse ACAAAGGCTCGGCATCAG				(*Alu* I)
	SNP7	Forward CATGCACCTCACAATCAACC	186	60	35	RFLP
	rs3767086	Reverse AGCTGACCTGGGTTCCTACA				(*Hae* III)
	SNP8	Forward GAGCTCCTGCATCCTCAAAG	196	60	35	RFLP
	rs554445	Reverse CCAAGGAAAGCGAGTTCAAG				(*Pst* I)
	SNP9	Forward GATCTCCATGCTGCCTTTTC	386	55	35	DS
	rs1160751	Reverse TCAAGTGTCCCATCAATTCG				
	SNP10	Forward CCAGAACCATCCAACGAAGT	325	58	35	DS
	rs1334804	Reverse ATCTCAGGCACAAAGGGTGT				
	SNP11	Forward CCAGTTGGGATGGTGGTG	235	63	35	DS
	rs3767067	Reverse ATTATGCACAGCCAGGAGGA				
	SNP12	Forward GAGGGTTCCAGTTGCTGTTT	288	60	35	DS
	rs550250	Reverse GCGTAGTCGGGGTAGAGGTT				
	SNP13	Forward AGAGATGCTGCCCTTCACAC	308	58	30	RFLP
	rs565537	Reverse AACTGCCTTTCAACCAGGAG				(*Hha* I)
	SNP14	Forward ATAGCCTCCCTCTCCTCCAA	210	60	35	DS
	rs3767048	Reverse GCACCCATGCATACACTCAC				
	SNP15	Forward TCACTTCCTGACCCTGTCTTC	196	55	40	DS
	rs3767045	Reverse CTCTGGGCTTTGGCTGTT				
	SNP16	Forward CTCCCTTGAGGCGTGTTTT	182	58	40	DS
	rs2993076	Reverse AAGGACAGACCCCACATCCT				
GRIK4	SNP1	Forward CTGCAGTCTTTCTGCTGCAC	253	58	40	DS
	rs2248404	Reverse GGAAGCGCTAGATAGGTCGT				
	SNP2	Forward TCAAAGAGGGTTCCAGGATG	252	58	30	DS
	rs1317176	Reverse AGCAGACATAGCCCTGCTGT				
	SNP3	Forward AGCTGACACACTCCCTCACC	184	55	35	DS
	rs1317514	Reverse GCAAGCGTATAGAGGGGAAA				
	SNP4	Forward GCCTCGTTCCTTGACTGTGT	231	58	30	DS
	rs1343789	Reverse GGGCTCACCAAGGTCAGTAT				
	SNP5	Forward CAGAGACCCTCTGAGGTTGG	393	58	30	DS
	rs1893906	Reverse AATCACTTGAACCGGCAGAC				
	SNP6	Forward GGAACCAGACTGCTCGGATA	381	58	30	DS
	rs2000870	Reverse CCCTAGACAAGGTGGGGATT				
	SNP7	Forward CCTCTGAGACCTGCAGCAAT	247	63	30	DS
	rs2000868	Reverse CACCAGGCTGGAGCAGATAC				
	SNP8	Forward GGGAAAGGAAGGCTCAATTC	363	58	30	DS
	rs3133855	Reverse GCTATTGAAAGGCTGGGAGA				
	SNP9	Forward GGAGGCATCCATATTCAGGA	265	60	30	DS
	rs3133855	Reverse TGGATTCTCCCTCCTTCCTT				
	SNP10	Forward CAAGACAGCATTTTGGAGCA	283	58	30	DS
	rs3133226	Reverse TCCTCTTGGGAACTGTGAGG				

Table 1 (*continued*)

Gene	SNP	Primer sequence (5′-3′)	Product size (bp)	Annealing temp (°C)	Cycle number	Typing method[a]
	SNP11	Forward GCCCATGTCCTGAGTGAAAG	338	58	30	DS
	rs3133845	Reverse CCTCATGACTGCTTCCTTGC				
	SNP12	Forward GACTCAGGGACCCCAAGAA	242	58	30	DS
	rs2852230	Reverse ACCAGCCAGGAAAACATGAC				
	SNP13	Forward TGAGGGTGCTGCTCTAACAA	253	58	30	DS
	rs2846092	Reverse TCATTGGGATGGCCTCTATC				
	SNP14	Forward AGTTGCAACCTTGGCTCACT	352	63	40	DS
	rs2846103	Reverse CTTCCAGAGACCACCCTCAC				
	SNP15	Forward CTCCTGACCTCATGATCTGC	267	65	40	DS
	rs4359220	Reverse AGAGATCACGTGCTGCTGAC				
	SNP16	Forward CTGGGCTATCCCTGCCTAGA	345	63	30	DS
	rs4936552	Reverse ACAGACTTGGGTTTGCATCC				
	SNP17	Forward GATGGGGAATGATGCTGTTC	245	69	30	DS
	rs2004676	Reverse ATTCAGTGGGACGAGACAGG				
	SNP18	Forward ATCCACAAGGCTGTCCATTC	317	58	30	DS
	rs3824978	Reverse CCACTAGGCACATGGCTTTT				
	SNP19	Forward TTCTCCTACTCCAGGCCAAG	322	58	30	DS
	rs3802911	Reverse TTACCTGCCGAAAACCATTC				
	SNP20	Forward CCTGGGCTGAAATGATTGAT	222	58	30	DS
	rs2156637	Reverse TTTCCTGAACACCCGACAAT				
	SNP21	Forward AGCCTGGCTGAGTCCACTT	220	58	30	DS
	rs2156635	Reverse GGACAGGCAGACAAAGAAGG				
	SNP22	Forward CACCCCAAAGTAGCCCATTA	247	63	30	DS
	rs2156634	Reverse ATCTCTGCCAGCTCCTTGAG				
	SNP23	Forward GACAGGGAAGGGAAGAGGAG	258	65	30	DS
	rs2298725	Reverse ACCCACAGAAATGGGAACTG				
	SNP24	Forward TTGCCAGCCAGCTCTCTTAT	246	58	30	DS
	rs611065	Reverse GGAGGGCCACAAATGAGTCT				
GRIK5	SNP1	Forward GGAGGAGGGGAAGGATATGA	261	58	30	DS
	rs1056995	Reverse CCTTTCCCACTCAGAGACCA				
	SNP1.5	Forward CAGAGCCAGAAGTGGGAGAC	233	58	30	DS
	hCV1854167	Reverse CAGTGTCTCCTCCCCATCAC				
	SNP2	Forward TGTACTCCGAGCTTCCCAAG	330	60	30	DS
	rs4803523	Reverse GAACACTGGAGGAGGAGCTG				
	SNP4	Forward TGGTCTGAACAATGGGGAAT	229	58	30	DS
	rs8099939	Reverse CGAGTGGAGTTGCTGTCAGA				
	SNP5	Forward CTTCTGCAGCAAAGGAGGTT	186	60	30	DS
	rs4803520	Reverse GCCAAGGTGTCAAGAGAAGG				

[a] RFLP: restriction fragment length polymorphism; DS: direct sequencing.

SNP23 are synonymous SNPs located within exons 9, 11 and 13, respectively. The other 19 SNPs are located in noncoding regions (Fig. 1b).

2.2.3. *GRIK5*

After the examination of 46 SNPs from the database, we selected the following five common SNPs distributed in the *GRIK5* region spanning over 90 kb: SNP1, rs1056995; SNP 2, hCV1854167; SNP3, rs4803523; SNP4, rs8099939; SNP5, rs4803520. All SNPs are located in noncoding regions (Fig. 1c).

2.3. Genotyping of case–control pairs

All genotypes were determined either by direct sequencing or by polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) as previously described (Shibata et al., 2001). Table 1 shows the nucleotide sequences of each primer, PCR conditions and genotyping methods for the 45 SNPs in the three gene regions. The raw data of direct sequencing were compiled on PolyPhred (Nickerson et al., 1997). The overall error rate was < 1%, estimated by compar-

Table 2
Genotype and allele frequencies of SNPs in *GRIK3*, *GRIK4*, and *GRIK5*

Polymorphism	Genotype count			P^a	Allele frequency (%)		P^b
a. *GRIK3*							
SNP1	AA	AT	TT		A	T	
Cases	3	21	76	0.3344	13.5	86.5	0.4420
Controls	0	21	79		10.5	89.5	
SNP2	AA	AG	GG		A	G	
Cases	37	44	19	0.8958	59	41	0.6828
Controls	39	45	16		61.5	38.5	
SNP3	AA	AC	CC		A	C	
Cases	78	18	4	0.1116	87	13	0.7606
Controls	77	23	0		88.5	11.5	
SNP4	CC	CT	TT		C	T	
Cases	1	13	86	0.4108	7.5	92.5	0.3004
Controls	1	20	79		11	89	
SNP5	CC	CT	TT		C	T	
Cases	1	15	84	0.4940	8.5	91.5	0.3227
Controls	1	22	77		12	88	
SNP6	AA	AG	GG		A	G	
Cases	12	45	43	0.0615	34.5	65.5	0.0319
Controls	19	53	28		45.5	54.5	
SNP7	GG	GT	TT		G	T	
Cases	77	22	1	0.4408	88	12	>0.9999
Controls	80	17	3		88.5	11.5	
SNP8	CC	CG	GG		C	G	
Cases	9	45	46	0.1204	31.5	68.5	0.0610
Controls	18	46	36		41	59	
SNP9	AA	AG	GG		A	G	
Cases	79	18	3	0.6886	88	12	0.8756
Controls	79	20	1		89	11	
SNP10	CC	CT	TT		C	T	
Cases	2	22	76	>0.9999	13	87	0.8800
Controls	1	22	77		12	88	
SNP11	CC	CT	TT		C	T	
Cases	84	15	1	0.8509	91.5	8.5	0.7304
Controls	81	18	1		90	10	
SNP12	CC	CT	TT		C	T	
Cases	1	16	83	>0.9999	9	91	>0.9999
Controls	1	17	82		9.5	90.5	
SNP13	AA	AG	GG		A	G	
Cases	4	19	77	0.4608	13.5	86.5	0.6505
Controls	1	21	78		11.5	88.5	
SNP14	AA	AG	GG		A	G	
Cases	66	30	4	0.3496	81	19	0.1331
Controls	76	22	2		87	13	
SNP15	GG	GT	TT		G	T	
Cases	86	14	0	0.6522	93	7	0.5809
Controls	84	14	2		91	9	
SNP16	CC	CT	TT		C	T	
Cases	39	49	12	0.1949	63.5	36.5	0.2439
Controls	51	37	12		69.5	30.5	
b. *GRIK4*							
SNP1	AA	AG	GG		A	G	
Cases	6	40	54	0.9002	26	74	0.7356
Controls	7	42	51		28	72	

Table 2 (*continued*)

Polymorphism	Genotype count			P^a	Allele frequency (%)		P^b
b. *GRIK4* (*continued*)							
SNP2	AA	AG	GG		A	G	
Cases	5	31	64	0.9081	20.5	79.5	0.8018
Controls	4	30	66		19	81	
SNP3	AA	AG	GG		A	G	
Cases	34	51	15	0.8535	59.5	40.5	0.6822
Controls	37	50	13		62	38	
SNP4	CC	CT	TT		C	T	
Cases	16	48	36	0.7434	40	60	0.8848
Controls	12	52	36		38	62	
SNP5	AA	AG	GG		A	G	
Cases	5	42	53	>0.9999	26	74	>0.9999
Controls	6	41	53		26.5	73.5	
SNP6	CC	CG	GG		C	G	
Cases	16	43	41	0.3552	37.5	62.5	0.2220
Controls	19	50	31		44	56	
SNP7	CC	CT	TT		C	T	
Cases	15	43	42	0.6967	36.5	63.5	0.5371
Controls	16	48	36		40	60	
SNP8	CC	CT	TT		C	T	
Cases	39	40	21	>0.9999	59	41	>0.9999
Controls	39	39	22		58.5	41.5	
SNP9	AA	AC	CC		A	C	
Cases	61	32	7	0.9750	77	23	>0.9999
Controls	61	31	8		76.5	23.5	
SNP10	CC	CT	TT		C	T	
Cases	31	49	20	0.8091	55.5	44.5	0.9200
Controls	32	45	23		54.5	45.5	
SNP11	AA	AG	GG		A	G	
Cases	27	52	21	0.6404	53	47	0.4839
Controls	25	48	27		49	51	
SNP12	CC	CG	GG		C	G	
Cases	27	52	21	0.1749	53	47	0.1334
Controls	23	44	33		45	55	
SNP13	AA	AG	GG		A	G	
Cases	16	49	35	0.3300	40.5	59.5	0.2559
Controls	9	51	40		34.5	65.5	
SNP14	CC	CT	TT		C	T	
Cases	4	35	61	0.4863	21.5	78.5	0.5321
Controls	5	27	68		18.5	81.5	
SNP15	AA	AG	GG		A	G	
Cases	65	33	2	0.2821	81.5	18.5	0.2782
Controls	72	28	0		86	14	
SNP16	AA	AT	TT		A	T	
Cases	31	49	20	0.6619	55.5	44.5	0.4228
Controls	26	50	24		51	49	
SNP17	CC	CT	TT		C	T	
Cases	25	48	27	0.9826	49	51	0.9203
Controls	25	46	29		48	52	
SNP18	AA	AG	GG		A	G	
Cases	12	38	50	0.2587	31	69	0.8300
Controls	8	49	43		32.5	67.5	
SNP19	CC	CT	TT		C	T	
Cases	26	58	16	0.1949	55	45	0.1094
Controls	17	59	24		46.5	53.5	

(*continued on next page*)

Table 2 (*continued*)

Polymorphism	Genotype count			P^a	Allele frequency (%)		P^b
b. *GRIK4* (*continued*)							
SNP20	AA	AG	GG		A	G	
Cases	23	50	27	0.7837	48	52	>0.9999
Controls	25	45	30		47.5	52.5	
SNP21	CC	CG	GG		C	G	
Cases	51	45	4	0.1180	73.5	26.5	0.6454
Controls	60	32	8		76	24	
SNP22	AA	AG	GG		A	G	
Cases	82	16	2	0.8848	90	10	0.7304
Controls	84	15	1		91.5	8.5	
SNP23	CC	CT	TT		C	T	
Cases	57	35	8	0.1991	74.5	25.5	0.1237
Controls	44	46	10		67	33	
SNP24	AA	AC	CC		A	C	
Cases	45	36	19	0.4286	63	37	0.6014
Controls	45	42	13		66	34	
c. *GRIK5*							
SNP1	CC	CT	TT		C	T	
Cases	79	19	2	0.7110	88.5	11.5	0.5491
Controls	75	22	3		86	14	
SNP2	CC	CG	GG		C	G	
Cases	10	37	53	0.7536	28.5	71.5	0.8233
Controls	7	40	53		27	73	
SNP3	CC	CT	TT		C	T	
Cases	61	31	8	0.3425	76.5	23.5	0.3943
Controls	64	33	3		80.5	19.5	
SNP4	GG	GT	TT		G	T	
Cases	39	41	20	0.9652	59.5	40.5	>0.9999
Controls	38	43	19		59.5	40.5	
SNP5	AA	AG	GG		A	G	
Cases	0	15	85	0.6796	7.5	92.5	>0.9999
Controls	1	12	87		7	93	

A significant *P* value is underlined.

[a] Fisher's exact probability tests, case vs. control (2 × 3, two-tailed).

[b] Fisher's exact probability tests, case vs. control (2 × 2, two-tailed).

ing the same samples genotyped by two different persons using direct sequencing and RFLP, respectively (data not shown).

2.4. Statistics

To control genotyping errors, Hardy–Weinberg equilibrium was checked in controls by the two-tailed χ^2 test ($df=1$). Statistical differences in genotype and allele frequencies between schizophrenic and control subjects were evaluated with Fisher's exact probability test (2 × 3 and 2 × 2, respectively). The normalized linkage disequilibrium statistic D' was calculated using haplotype frequencies estimated by the EH program, version 1.14 (Xie and Ott, 1993). Statistical analysis for the haplotype association was done by the two-tailed χ^2 test ($df=3$) according to Sham (1998). We excluded combinations of SNPs showing very weak LD ($D'<0.3$) from the haplotype analyses. The significance level for all statistical tests was 0.05. We applied the Bonferroni correction for all multiple tests.

3. Results

3.1. Single marker association analysis

We determined genotype and allele frequencies of the total 45 common SNPs in the three gene regions,

i.e., *GRIK3*, *GRIK4* and *GRIK5* (Table 2). The average allele frequencies of the SNPs are 0.18, 0.35 and 0.22, respectively. Given the equivalent frequency for the susceptible allele, the expected detection powers for *GRIK3*, *GRIK4* and *GRIK5* are 0.70 to 0.84, 0.84 to 0.94, and 0.75 to 0.89, respectively, under the multiplicative model with genotype relative risk=1.8 to 2.0 (Ohashi and Tokunaga, 2001). Genotype frequencies of any of the 45 SNPs in controls did not show significant deviations from Hardy–Weinberg equilibrium (data not shown). Only the allele frequency of SNP6 in *GRIK3* showed a significant association with disease status (P=0.0319, Fisher's exact probability test). The nominal significance of the finding, however, did not survive the Bonferroni correction (n=16). We also failed to replicate the significance in the Aichi set (P=0.686, Fisher's exact probability test, data not shown).

3.2. Pairwise LD analysis

LD around common alleles can be measured with a modest sample size of 40–50 individuals to a precision within 10%–20% of the asymptotic limit (Reich et al., 2001). To evaluate the magnitude of LD, we calculated D' from the haplotype frequencies estimated by the EH program for all possible pairs of the tested SNPs within each gene region. Fig. 2 shows LD patterns within each gene region visualized by the GOLD program (Abecasis and Cookson, 2000). There was no essential difference in LD patterns in any of the genes between cases and controls. Since we selected more SNPs in low-LD subregions, we were able to localize several small subregions where LD drops abruptly. In the *GRIK3* region, we found one such LD gap between SNP14 and SNP15 (10.4 kb, D'=0.0166). In the *GRIK4* region, we found three gaps: SNP9–SNP10 (2.9 kb, D'=0.2023), SNP14–SNP15 (6.7 kb, D'=0.091), and SNP20–SNP21 (0.9 kb, D'=0.1800). No clear LD gaps were observed in the *GRIK5* region.

3.3. Haplotype association analysis

We intended to test haplotype associations with schizophrenia for all possible pairs of SNPs where

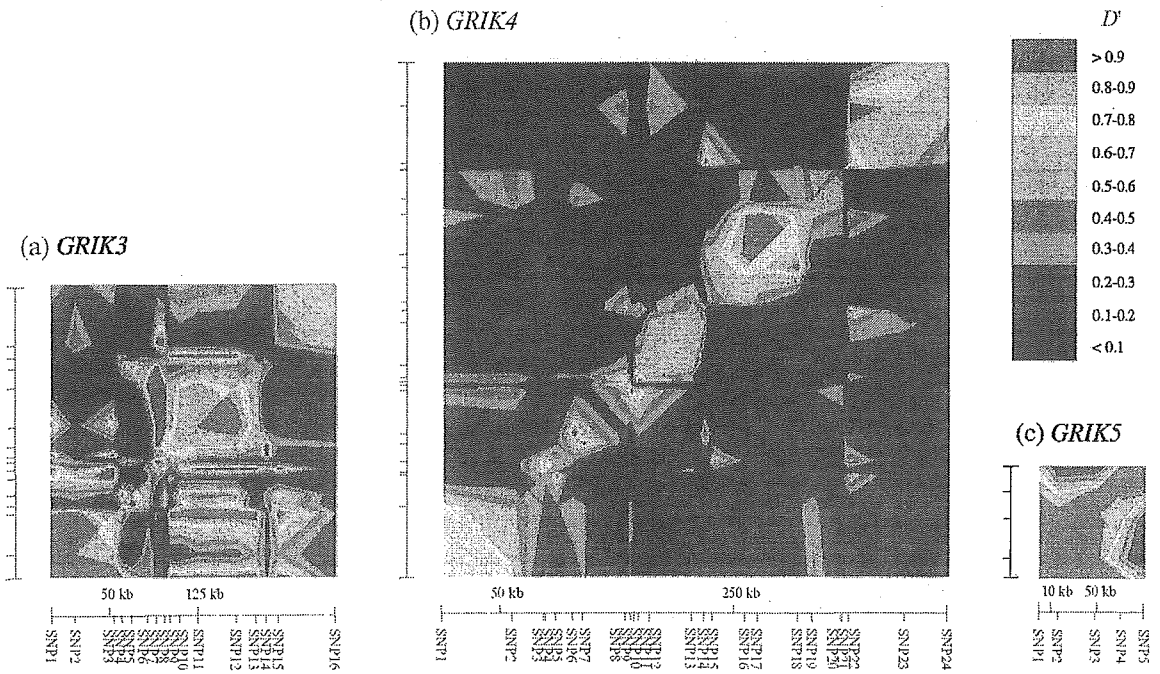

Fig. 2. LD patterns in the three gene regions of *GRIK3* (a), *GRIK4* (b) and *GRIK5* (c). The magnitude of LD (D') was visualized by the GOLD program (Abecasis and Cookson, 2000). The composite LD patterns in cases (upper diagonal) and in controls (lower diagonal) were drawn as squares in scale.

LD was maintained. To avoid the inflation of the number of statistical tests, we excluded 32, 141 and 1 SNP pairs from further analyses of *GRIK3*, *GRIK4* and *GRIK5*, respectively, because of very weak LD ($D'<0.3$) in both cases and controls. Out of a possible 120 pairs, we analyzed 88 pairs of SNPs showing moderate to strong LD in *GRIK3*. The following seven pairs showed significant haplotype associations with the disease: SNP1–SNP14 ($P=0.0391$, $df=3$, χ^2 test), SNP2–SNP6 ($P=0.0402$, $df=3$, χ^2 test), SNP3–SNP6 ($P=0.0166$, $df=3$, χ^2 test), SNP4–SNP6 ($P=0.0338$, $df=3$, χ^2 test), SNP5–SNP6 ($P=0.0196$, $df=3$, χ^2 test), SNP8–SNP14 ($P=0.0056$, $df=3$, χ^2 test), and SNP8–SNP15 ($P=0.0064$, $df=3$, χ^2 test) (Table 3a). However, none of the significant associations survived Bonferroni corrections ($n=88$). We also tested the most significant pair (SNP8–SNP14 of *GRIK3*) in the Aichi set, which failed to replicate the significance ($P=0.494$, $df=3$, χ^2 test; data not shown). We analyzed 90 pairs of SNPs out of 231 possible pairs in *GRIK4*. The following four pairs showed significant haplotype associations with the disease: SNP1–SNP19 ($P=0.0432$, $df=3$, χ^2 test), SNP2–SNP19 ($P=0.0242$, $df=3$, χ^2 test), SNP3–SNP4 ($P=0.0007$, $df=3$, χ^2 test), and SNP6–SNP19 ($P=0.0460$, $df=3$, χ^2 test) (Table 3b). Again none of the significant associations survived the Bonferroni correction ($n=90$). We also tested the most significant pair (SNP3–SNP4 of *GRIK4*) in the Aichi set, which failed to replicate the significance ($P=0.155$, $df=3$, χ^2 test; data not shown). We analyzed nine pairs of SNPs out of a possible 10 pairs in *GRIK5*. None of them showed significant haplotype associations with the disease (Table 3c).

4. Discussion

Our general approach to the testing of haplotype associations is based on haplotyping neighboring SNPs that are in moderate LD. We first genotyped both cases and controls for the SNPs evenly spaced. When insufficient LD was observed in the neighboring SNP pair ($D'<0.3$), additional SNPs were selected for genotyping within the LD gap. In other words, the candidate gene region is divided into multiple subregions where moderate LD is observed. Instead of haplotyping the entire gene region with many SNPs at once, we examined each subregion by haplotyping SNPs within the subregion so that we extensively tested the association of the entire gene with the disease (Shibata et al., 2002; Takaki et al., 2004). Through the SNP validation and LD analysis process, we selected 16, 24 and 5 common SNPs in the regions of *GRIK3*, *GRIK4* and *GRIK5* (Fig. 1). The average intervals of the SNPs were 16.1, 18.8 and 22.8 kb,

Table 3a
Pairwise haplotype association of SNPs in *GRIK3* with schizophrenia

	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11	SNP12	SNP13	SNP14	SNP15
SNP2	0.840														
SNP3	0.216	0.339													
SNP4	0.451	–	0.602												
SNP5	0.552	0.486	–	0.687											
SNP6	0.121	0.040	0.017	0.034	0.020										
SNP7	0.632	0.767	0.849	–	–	0.166									
SNP8	0.192	0.231	–	0.071	0.076	–	0.200								
SNP9	0.787	–	0.948	–	–	0.061	0.598	0.255							
SNP10	0.641	–	0.710	0.227	0.255	–	0.978	0.245	0.989						
SNP11	0.792	–	0.932	0.306	0.342	–	0.964	0.084	0.835	0.753					
SNP12	0.433	–	0.457	0.581	0.619	0.066	0.998	0.104	0.944	0.914	0.173				
SNP13	0.134	0.494	0.120	0.457	0.472	0.070	0.932	0.239	–	0.214	0.225	0.472			
SNP14	0.039	–	–	0.268	0.277	–	0.334	0.006	0.389	0.385	0.279	0.257	0.362		
SNP15	0.457	0.854	0.593	0.407	0.266	–	0.906	0.006	–	0.696	–	–	–	–	
SNP16	0.121	–	0.070	–	–	–	–	–	0.216	0.560	0.423	0.606	–	–	0.577

P values by the two-tailed χ^2 test for two-way haplotype association ($df=3$).
SNP pairs showing insufficient LD ($D'<0.3$) were not tested (denoted as "–").
Significant *P* values are underlined.

Table 3b
Pairwise haplotype association of SNPs in *GRIK4* with schizophrenia

	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11	SNP12	SNP13	SNP14	SNP15	SNP16	SNP17	SNP18	SNP19	SNP20	SNP21	SNP22	SNP23
SNP2	0.706																						
SNP3	–	0.854																					
SNP4	0.249	–	0.001																				
SNP5	–	–	0.259	0.052																			
SNP6	–	0.136	–	–	0.568																		
SNP7	0.593	0.410	–	–	–	0.063																	
SNP8	0.245	0.532	–	–	–	–	–																
SNP9	0.116	0.094	–	–	0.572	–	–	0.706															
SNP10	0.286	0.520	–	–	–	–	–	0.849	–														
SNP11	0.200	0.457	–	–	0.272	0.516	0.821	0.564	–	0.552													
SNP12	–	–	–	–	–	–	–	0.184	–	0.339	0.430												
SNP13	–	–	–	0.334	–	–	–	–	–	0.540	0.505	0.376											
SNP14	–	–	–	–	–	0.323	0.308	0.192	0.734	0.193	0.365	–	0.451										
SNP15	–	–	–	–	0.606	–	–	0.323	–	–	–	–	0.212	–									
SNP16	–	–	–	–	0.811	–	–	–	–	–	–	–	–	0.401	0.593								
SNP17	–	–	–	–	–	–	–	–	–	–	–	–	–	0.163	0.430	0.089							
SNP18	0.440	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.410	0.062						
SNP19	0.043	0.024	–	–	–	0.046	0.159	–	0.079	–	–	–	–	–	–	0.135	–	0.291					
SNP20	–	0.687	–	–	–	–	–	0.509	0.070	–	–	–	–	0.878	–	0.787	–	0.964	0.193				
SNP21	–	–	0.682	–	0.873	0.207	0.437	–	–	–	–	–	–	–	–	–	–	0.451	–	–			
SNP22	–	–	0.286	–	0.739	–	–	–	–	–	0.701	–	0.275	0.806	0.461	–	–	0.878	–	–	0.437		
SNP23	–	0.100	–	–	–	–	–	0.125	–	0.179	–	0.058	0.095	–	–	–	–	–	–	–	–	0.289	
SNP24	–	–	–	–	–	–	–	0.109	–	–	–	0.085	–	–	–	–	–	–	–	–	–	–	0.417

P value by the two-tailed χ^2 test for two-way haplotype association ($df=3$).
SNP-pairs showing insufficient LD ($D'<0.3$) were not tested (denoted as "–").
Significant *P* values are underlined.

Table 3c
Pairwise haplotype association of SNPs in *GRIK5* with schizophrenia

	SNP1	SNP2	SNP3	SNP4
SNP2	0.896			
SNP3	0.739	0.619		
SNP4	0.479	0.407	0.479	
SNP5	0.724	–	0.577	0.999

P values by the two-tailed χ^2 test for two-way haplotype association ($df=3$).
SNP-pairs showing insufficient LD ($D'<0.3$) were not tested (denoted as "–").

respectively. Although we observed a nominal significance in the allele frequencies of SNP6 in *GRIK3* ($P=0.0319$, Fisher's exact probability test), there was no significant association observed in any of the other SNPs tested (Table 2). The significance of SNP6 in *GRIK3* was lost when we applied the Bonferroni correction ($n=16$). We also failed to replicate the significance in our second sample set, the Aichi set ($P=0.686$, Fisher's exact probability test). Therefore, we concluded that there is no common SNP showing a "single marker" association with schizophrenia in the 45 common SNPs of the three gene regions. We also examined an exonic SNP in *GRIK3*, rs6691840, for which a significant association with schizophrenia has been reported in the Italian population (Begni et al., 2002). Although we examined an equivalent sample size (100 cases and 100 controls) as in the study by Begni et al. (2002) (99 cases and 116 controls), the SNP was very rare (MAF=0.02) in our controls and completely monomorphic in our cases, indicating that there is no detectable association of the SNP with schizophrenia in the Japanese population. As we examined all of the exonic SNPs available on the database, there were no common exonic SNPs left untested for the association with the disease.

Seven pairs of SNPs in *GRIK3* and four pairs of SNPs in *GRIK4* showed significant haplotype associations with schizophrenia ($P=0.0007$, $df=3$, χ^2 test; see Tables 3a and b). However, none of them remained significant after Bonferroni correction. In addition, we failed to replicate the most significant pairs of SNPs, SNP8–SNP14 of *GRIK3* ($P=0.494$, $df=3$, χ^2 test) and SNP3–SNP4 of *GRIK4* ($P=0.155$, $df=3$, χ^2 test) in our second sample set, the Aichi set. We conclude that there is no significant haplotype association in any of the three regions, *GRIK3*, *GRIK4* and *GRIK5*.

We analyzed pairwise LD within each gene region (Fig. 2a–c). We observed no essential difference in LD patterns between cases and controls in any of the gene regions. Although we sampled SNPs more densely in weak LD subregions, we still observed four LD gaps. In the *GRIK3* region, one such subregion was found between SNP14 and SNP15 (10.4 kb, $D'=0.017$) where two exons, exon 8 and exon 9 as well as the entire intron 8, are located. Although we examined all other SNPs reported on the database within this subregion, none showed a high enough frequency for statistical testing (>10%). Therefore, we could not analyze this subregion any further. In the *GRIK4* region, we found three gaps: SNP9–SNP10 (2.9 kb, $D'=0.202$), SNP14–SNP15 (6.7 kb, $D'=0.091$), and SNP20–SNP21 (0.9 kb, $D'=0.180$). The first two subregions are located within intron 1. The third subregion is located within intron 10. Although we tested all SNPs within the subregions reported on the database, none showed sufficient polymorphisms for statistical testing (>10%). Therefore, we could not study these potential hot spots for recombination any further.

The remaining regions, which are the vast majority of the three genes, were successfully covered by LD as we intended. Assuming that no common SNPs were left untested in the LD gaps, those might have escaped from our haplotype analyses, there is no common variant of the three kainate receptor genes that is significantly associated with schizophrenia. We conclude that the three kainate receptor genes, *GRIK3*, *GRIK4* and *GRIK5*, do not play a major role in schizophrenia pathogenesis in Japanese.

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ORIGINAL ARTICLE

Haplotype association between $GABA_A$ receptor γ2 subunit gene (GABRG2) and methamphetamine use disorder

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ABSTRACT

Psychostimulant use disorder and schizophrenia have a substantial genetic basis. Evidence from human and animal studies on the involvement of the γ-aminobutyric acid (GABA) system in methamphetamine (METH) use disorder and schizophrenia is mounting. As we tested for the association of the human $GABA_A$ receptor gamma 2 subunit gene (GABRG2) with each diagnostic group, we used a case–control design with a set of 178 subjects with METH use disorder, 288 schizophrenics and 288 controls. First, we screened 96 controls and identified six SNPs in GABRG2, three of whom we newly reported. Next, we selected two SNPs, 315C>T and 1128+99C>A, as representatives of the linkage disequilibrium blocks for further case–control association analysis. Although no associations were found in either allelic or genotypic frequencies, we detected a haplotypic association in GABRG2 with METH use disorder, but not with schizophrenia. This finding partly replicates a recent case–control study of GABRG2 in METH use disorder, and thus indicates that GABRG2 may be one of the susceptibility genes of METH use disorder.

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Keywords: $GABA_A$ γ2 subunit gene; methamphetamine; substance use disorder; polymorphism; haplotype; schizophrenia

INTRODUCTION

In recent years there has been a pronounced increase in use of psychostimulants involving methamphetamine (METH).[1] Lifetime prevalence of psychostimulant use in some developed countries is found in 1–3% of the adult population,[2] and psychostimulant use in any form may lead to abuse or dependence with physiological, psychological and behavioral component.[3] Findings from family and twin studies suggest that the genetic contribution is important for the development of psychostimulant use disorders. Heritability estimates from a population-based twin study for METH use disorder are substantial,[4,5] for example, 66% for psychostimulant abuse.[6]

The dopamine system is a prime candidate for genetic influence on drug abuse, particularly METH abuse, because it is thought to be involved in the reward and reinforcing mechanism in the meso-cortico-limbic system in the nucleus accumbens.[7] Moreover, the primary site of biological activity of METH is the dopamine transporter in this system.