Table 1. Association analyses of the AKT1 gene

	Multi-SNP haplotype systems						Genotypic distribution					
							M/M		M/m		m/m	
SNP ID	1 SNP	2 SNP	3 SNP	4 SNP	5 SNP	6 SNP	Meth	Control	Meth	Control	Meth	Control
SNP1 (rs3803300G>A)	0.15						63	124	91	234	28	79
		0.22										
SNP2 (rs1130214G>T)	0.97		0.096				128	315	51	108	3	14
		0.27		**0.0023**								
SNP3 (rs3730358C>T)	**0.019**		0.43		**0.0082**		136	364	43	68	3	5
		0.12		0.23		**0.017** (0.10)						
SNP4 (rs2498799G>A)	0.81		0.11		0.16		40	121	98	211	44	105
		0.53		0.063								
SNP5 (rs2494732A>G)	0.59		0.19				86	212	79	192	17	33
		0.16										
SNPA (rs2498804T>G)	0.20						63	142	92	206	27	89
Permutation *p* value	*0.097*	*0.40*	*0.28*	***0.0083*** (***0.0498***)	***0.023*** (*0.14*)							

p values were calculated by log likelihood ratio test (SNP1, allele-wise association; SNP2-6, global haplotypic association).
M, major allele; m, minor allele; Meth, methamphetamine-use disorder.
Bold values represent significant *p* values.
Values within parentheses represent *p* values after Bonferroni correction.

Table 2. Haplotype frequencies from positive permutation analysis

Combination of SNPs	Marker haplotype	Frequency		*p* values
		Meth	Control	
SNP1-2-3-4	A-G-C-G	0.28	0.20	0.023
	A-G-T-A	0.074	0.048	0.049
	G-G-C-G	0.12	0.21	0.0032

Meth, Methamphetamine-use disorder.

Discussion

A positive association between a SNP and AKT1 haplotypes was found in our Japanese Meth-use disorder samples. In assessing the components of these associations, we considered SNP3 to be a main component associated with Meth-use disorder, because the single marker association of SNP3 was significant in total samples ($p=0.019$). Interestingly, this SNP was associated with US schizophrenia in an original TDT analysis (Emamian et al., 2004). On the contrary, we found no association SNP3 to Japanese schizophrenia in a previous study (SNP5 was associated with Japanese schizophrenia) (Ikeda et al., 2004). This difference in predisposing SNPs between Japanese Meth-use disorder and Japanese schizophrenia might be explained by their respective linkage disequilibrium (LD) patterns. We have shown that the LD pattern in schizophrenia was slightly different from that in controls, while the pattern in Meth-use disorder tended to be similar to that in control samples (data not shown). These findings indicate that different predisposing polymorphisms may exist independently in schizophrenia and Meth-use disorder, and may be located in LD with SNP5 or SNP3 respectively.

The result of explorative analysis might support the 'gender effects' of Meth-use disorder, reported in a previous genetic association study of Meth-use disorder (Lin et al., 2003). Especially, female samples of

Table 3. Explorative analysis of gender effects

		Multi-SNP haplotype systems					
Gender	SNP ID	1 SNP	2 SNP	3 SNP	4 SNP	5 SNP	6 SNP
Female ($n=36$)	SNP1	0.55					
			0.41				
	SNP2	0.084		**0.017**			
			0.012		**0.012**		
	SNP3	**0.0011**		**0.049**		**0.028**	
			0.028		0.084		**0.023**
	SNP4	0.50		0.076		0.057	
			0.67		0.16		
	SNP5	0.34		0.79			
			0.57				
	SNPA	0.73					
Male ($n=146$)	SNP1	0.23					
			0.13				
	SNP2	0.28		0.18			
			0.51		**0.036**		
	SNP3	0.11		0.47		0.053	
			0.26		0.41		**0.042**
	SNP4	0.58		0.19		0.22	
			0.55		0.078		
	SNP5	0.40		0.12			
			0.30				
	SNPA	0.50					

Bold values represent significant p values.

Meth-use disorder were strongly associated with a SNP and haplotypes of AKT1, while male samples were weakly associated. However, because the sample size of female subjects was small ($n=36$), a type I error might occur in this explorative analysis. Even assuming that there are no 'gender effects' of AKT1, the fact remains that AKT1 is associated with Meth-use disorder. In this case, these association analyses of total and divided samples indicate the following interpretations. (1) SNP3 might not be an 'actual' predisposing SNP by itself, nor be in perfect LD with 'actual' predisposing polymorphisms, because male samples with Meth-use disorder were not associated with SNP3 (only total or female samples were associated with it). (2) At least some haplotypes of AKT1 may play a possible role in the development of Meth-use disorder, because two haplotypes of AKT1, including the combination of SNP1-2-3-4 and SNP 1-2-3-4-5-A, are associated with Meth-use disorder both in divided samples and total samples.

Our results had several limitations in terms of interpreting positive associations. (1) The positive associations we detected might be due to type I error, possibly because of population stratification, an unmatched-gender sample and small sample size, described above. Larger sample size and genomic control would be required. (2) Type I error might also have occurred because of multiple testing. We corrected p values by applying a permutation procedure and Bonferroni correction in total samples. However, in individual haplotypic analysis or explorative analysis of gender effects, we did not apply each correction. At this time, an optimal method for resolving this problem (correction among global and individual haplotypic analysis, or explorative-subgroup analysis) has not yet been established. Greater knowledge of genetic models and more useful methods would be required to re-analyse these results. (3) The other confounding factors such as ascertainment bias can account for the apparent association in this study. For example, because subjects with Meth-induced psychosis consisted of the majority of our samples, this condition would be over-represented in our samples of Meth-use disorder. Further explanation is given in Nishiyama et al. (2005).

Our results indicate that AKT1 may play a role in the development of Meth-use disorder. Our findings also support the hypothesis that abnormalities in AKT1 might contribute to the pathophysiology of DA-dependent behaviour such as Meth-use disorder and schizophrenia. Further studies including mutation search to detect 'actual' predisposing polymorphisms and functional analysis of AKT1 (or its cascade) may open the way to elucidation of the pathophysiology of this condition.

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Statement of Interest

None.

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Journal of Neural Transmission

Association study of the frizzled-3 (FZD3) gene with schizophrenia and mood disorders

Short Communication

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Summary. Two research groups have recently reported a significant association between schizophrenia and genetic variants of Frizzled-3 (FZD3) gene. We examined a possible association in a Japanese sample of schizophrenia, bipolar disorder, unipolar depression and controls with four single nucleotide polymorphisms (SNPs), tested in previous reports. We failed to find significant association in the four SNPs or haplotype analysis. The FZD3 gene might not play a role in conferring susceptibility to major psychosis in our sample.

Keywords: FZD3, schizophrenia, mood disorder, association study, single nucleotide polymorphism (SNP).

Introduction

Schizophrenia is a complex genetic disorder characterized by disturbances of cognition, emotion and social functioning. This disease is believed to involve genetic abnormalities in developmental/plasticity related processes during a critical period in neuronal growth (Weinberger et al., 2001). Wnt signal transduction cascades have been implicated in a variety of neurodevelopmental processes, e.g. segmentation, central nervous system patterning, and cell divisions (Wodarz and Nusse, 1998). Wnt proteins signal via cell surface transmembrane receptors, termed frizzleds, which display many properties

characteristic of members of the superfamily of G-protein-coupled receptors (Wang and Malbon, 2004). The frizzled-3 (FZD3) gene, a member of frizzles, is located on chromosome 8p21, repeatedly suggested as a positive linkage locus for schizophrenia (Lewis et al., 2003; McGuffin et al., 2003). The FDZ3 gene consists of 8 exons and 7 introns, spanning approximately 70 Kb (Kirikoshi et al., 2000). In accordance with this, two research groups have recently reported a significant association between schizophrenia and the FZD3 gene in Japanese and Chinese samples (Katsu et al., 2003; Yang et al., 2003). We tried to replicate these findings in an independent Asian sample. Furthermore, we also examined the possible association between the FZD3 gene with mood disorders, since schizophrenia and mood disorders might share the genetic vulnerability (Berrettini, 2003).

Methods and materials

Subjects

Subjects were 427 patients with schizophrenia (221 males and 206 females with mean age of 44.2 years [SD 14.5]), 91 with bipolar disorder (40 and 51; 53.6 years [SD 14.8]), and 396 with major depression (155 and 241; 53.4 years [SD 16.1]) and 473 healthy controls (228 and 245; 36.1 years [SD 12.5]). All the subjects were biologically unrelated Japanese. Consensus diagnosis was made for each patient by at least two trained psychiatrists according to the DSM-IV criteria. Controls were healthy volunteers who had no current or past contact to psychiatric services. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by institutional ethical committees.

SNP genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. We genotyped four SNPs (single nucleotide polymorphisms; dbSNP accession: rs960914 in intron3, rs2241802 in exon5: A435G, L145L, rs2323019 in intron5 and rs352203 in intron5) in the FZD3 gene, which were examined in the previous two studies (Katsu et al., 2003; Yang et al., 2003). Genotyping was performed with the TaqMan 5′-exonuclease allelic discrimination assay, described previously (Hashimoto et al., 2004a, b). Briefly, primers and probes for detection of the SNPs are: rs960914: forward primer 5′-CTTTTATAAAGAAATTTGAAACAT CAGAACATGGGA-3′, reverse primer 5′-ACTTTTTCACTGCTTGGGAGTTATTCT-3′, probe 1 5′-VIC-CTGAATGGCTGCTATC-MGB-3′, and probe 2 5′-FAM-TCTGAATGGCTACTATC-MGB-3′; rs2241802: forward primer 5′-ATGAGCCATATCCTCGACTTGTG-3′, reverse primer 5′-GGACACCAAAAACCATAGTCTCTCT-3′, probe 1 5′-VIC-TCCAGCTAAATTCAG-MGB-3′, and probe 2 5′-FAM-CAGCCAAATTCAG-MGB-3′; rs2323019: forward primer 5′-GAAT TACTTTGTTTTTCTAGATTCTTGAATTGAAAGC-3′, reverse primer 5′-CCAACCTGGTTAA TAATGGTCTTTTGG-3′, probe 1 5′-VIC-TCATTTATTGTCAATGTTTTAA-MGB-3′, and probe 2 5′-TCATTTATTGTCAATATTTTAA-MGB-3′; rs352203: forward primer 5′-CCTGAAAAAA TATTCTATATCTCTTGTTTTGCCA-3′, reverse primer 5′-CAACCAGGACATAACAGTATTA CAGTTTCTAT-3′, probe 1 5′-VIC-TCCTTCATGTCGTATTC-MGB-3′, and probe 2 5′-FAM-TTTCCTTCATATCGTATTC-MGB-3′. PCR cycling conditions were: at 95°C for 10 minutes, 45 cycles of 92°C for 15 seconds and 60°C for 1 minute.

Statistical analysis

Statistical analysis of association studies was performed using SNPAlyse software (DYNACOM, Yokohama, Japan). The presence of Hardy-Weinberg equilibrium was examined using the $\chi\chi^2$ test for goodness of fit. Allele distributions between patients and controls were analyzed by the

Table 1. Allele distribution for SNPs in the FZD3 gene between major psychoses and controls

dbSNP ID	SNP	Controls	Schizophrenia		Bipolar		Unipolar	
		n = 473	n = 427	*P* value	n = 91	*P* value	n = 397	*P* value
rs960914	T/C	.398	.396	.91	.352	.23	.386	.61
rs2241802	G/A	.453	.458	.85	.451	.94	.455	.96
rs2323019	A/G	.407	.420	.57	.357	.21	.409	.91
rs352203	T/C	.397	.396	.97	.352	.24	.386	.64

χ^2 test for independence. The measures of linkage disequilibrium (LD), denoted as D', was calculated from the haplotype frequency using Expectation-Maximization algorithm. Case-control haplotype analysis was performed by the permutation method to obtain empirical significance (Good, 2000). The global p-values represent the overall significance using the χ^2 test when the observed versus expected frequencies of all the haplotypes are considered together. The individual haplotype was tested for association by grouping all others together and applying the χ^2 test with 1 df. P-values were calculated based on 10,000 replications. All p-values reported are two tailed. Statistical significance was defined at $p < 0.05$.

Results

The obtained allele frequencies for the patients and controls are shown in Table 1. The genotype distributions for all the diagnostic groups were in Hardy-Weinberg equilibrium (data not shown). There was no significant difference in genotype distributions or allele frequencies of the four SNPs in the FZD3 gene between the controls and any patient group, although previous studies reported positive associations between schizophrenia and several SNPs (Katsu et al., 2003; Yang et al., 2003). We computed the LD between the SNPs using D', which ranged between 0.8 and 1.0, indicating strong to intermediate LD between the markers. Adjacent combinations of up to four markers were tested, however, any haplotype combination was not significantly associated with any diagnostic group (all global p-values > 0.2).

Discussion

This study examined the possible association of the FZD gene with schizophrenia and mood disorders in our Japanese sample. We obtained no evidence for a significant association of the genetic variations of the FZD gene with any diagnostic group, suggesting that the examined polymorphisms play no major role in the pathogenesis of major psychoses in our sample. Our results are thus inconsistent with the results of the previous case-control study which reported a significantly higher frequency of the T allele of rs960914 in patients with schizophrenia than in controls (Katsu et al., 2003). The frequencies of the T allele were 0.62 and 0.51 in schizophrenics (n = 209) and controls (n = 200) in their Japanese sample, while the frequencies of the T allele were 0.60 and 0.60 in patients with schizophrenia (n = 427) and controls (n = 473) in ours. A highly significant association of the other three SNPs (rs2241802, rs2323019 and rs352203) and their three marker haplotypes with schizophrenia patients was reported in a family-based association study in a Chinese population

(Yang et al., 2003), while no evidence of such an association was obtained in our results (Chinese: global p-value < 0.000001, GAT haplotype p value < 0.000001; vs our results: global p-value $= 0.31$, GAT haplotype p-value $= 0.97$). Recently, the positive association between schizophrenia and the FZD3 gene has been reported in case-control study in a Chinese population (Zhang et al., 2004). This study presented that a new marker, rs880481, created the most positive results. Further analysis using this new marker should be examined in other ethnic populations.

A possible explanation for the discrepancy between the previous results and ours is a type II error in our sample. The odds ratio of the T allele of rs960914 was 1.54 in Japanese study (Katsu et al., 2003). However, power analysis revealed that our sample size could detect a significant association between the examined risk alleles (frequency of 0.4–0.6) and schizophrenia with a power of 90% when odds ratio was assumed to be 1.4 or more and the critical p-value was set at 0.05. There is only a small chance that a clinically meaningful difference would have been missed with the data. Secondly, it is possible that LD with other unknown polymorphisms, that is truly responsible for giving susceptibility to schizophrenia, may explain the discrepancy. Alternatively, the significant association observed by the previous two groups may have arisen by chance. The case-control association study is subject to the effect of population stratification, although the patients and controls were ethnically matched.

More recently, an extensive family-transmission and case-control analysis in a Japanese population with additional post-mortem mRNA expression data and family trio analysis in a British population yielded negative results for association between the FZD3 gene and schizophrenia (Ide et al., 2004; Wei and Hemmings, 2004), which is consistent with our results.

In conclusion, we obtained no evidence for an association between the FZD3 gene and schizophrenia or mood disorders, suggesting that this gene has no major role in conferring susceptibility to major psychoses in our sample.

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No association with the calcineurin A gamma subunit gene (PPP3CC) haplotype to Japanese schizophrenia

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Summary. Calcineurin, one of the serine/threonine protein phosphatase, comprises more than 1% of the total protein content in brain. This evidence points towards important roles of calcineurin in neural function. Miyakawa et al. reported that forebrain-specific calcineurin knockout mice showed the behavioral abnormalities that are often observed in schizophrenia patients. Based on this evidence, they suggested that calcineurin dysfunction could be involved in schizophrenia pathogenesis. Thereafter this report, Gerber et al. performed transmission disequilibrium test (TDT) studies and showed an evidence for a nominally significant over-transmission of a common haplotype of the human calcineurin A γ subunit gene (PPP3CC). We performed association analysis of PPP3CC in Japanese sample of 457 schizophrenia cases and 429 controls. To our regret, we could not confirm the association with Japanese schizophrenia to PPP3CC including core at-risk haplotype. Our result suggests that PPP3CC may not play a major role in Japanese schizophrenia.

Keywords: Single nucleotide polymorphism, calcineurin, haplotype.

Introduction

Phenotype of schizophrenia is characterized by positive and negative symptoms such as poor social function and cognitive dysfunction including attention deficit measured by pre-pulse inhibition test. Schizophrenia is also characterized by its fairly high heritability and abnormalities of glutamate (Moghaddam, 2003), γ-aminobutyric acid (GABA) (Wassef et al., 2003) and synaptic connectivity (Weinberger, 1999; Glantz and Lewis, 2001; Keshavan, 1999).

Recently, Miyakawa et al. reported that forebrain-specific calcineurin B subunit (CNB) knockout mice showed increased locomotor activity, decreased social interaction and impairments in pre-pulse inhibition similar to schizophrenic patients (Miyakawa et al., 2003). Calcineurin, one of the serine/threonine protein phosphatase, is highly enriched in neural tissue, as its concentration in brain is 10–20 times that found in other tissues and it comprises more than 1% of total protein content in the brain (Shibasaki et al., 2002; Klee et al., 1998; Wallace et al., 1980; Yakel, 1997; Guerini et al., 1990). Calcineurin also has been shown to have important roles in neural function. For example, activation of calcineurin inhibits the release of neurotransmitters, glutamate and GABA (Nichols et al., 1994; Greengard, 2001) and desensitization of the postsynaptic NMDA receptor (Tong, 1995; Lieberman, 1994). In addition, mice deficient in calcineurin-nuclear factor of activated T cells (NFAT) signaling had dramatic defects in axonal outgrowth (Graef et al., 2003). Considering the role of calcineurin in the glutamate and GABA system, in plasticity of neuron, and in social interaction and cognition, calcineurin would be a probable candidate gene for schizophrenia.

Thereafter Miyakawa's report, Gerber et al. performed transmission disequilibrium test (TDT) studies to examine the association of calcineurin-related candidate genes (calcineurin subunits, calcineurin binding proteins and proteins functionally coupled to calcineurin) with schizophrenia in 410 affected families collected from the United States (US) and South Africa (SA). They found an evidence for a nominally significant over-transmission of a common haplotype of the human calcineurin A γ subunit gene (PPP3CC) (Gerber et al., 2003) which is located within chromosome 8p21.3, a region that has been identified as schizophrenia susceptibility locus by linkage studies. Here, we performed a replication of their study with Japanese schizophrenia patients and controls.

Material and methods

Subjects

Subject consisted of 457 patients with schizophrenia (male: 242, female: 215) and 429 controls (male: 201, female: 228). All subjects were unrelated Japanese. All patients fulfilled the DSM-IV criteria for schizophrenia. The general characterization of these subjects and description of their psychiatric assessment were identical criteria published elsewhere (Suzuki et al., 2003). After description of the study, written informed consent was obtained from each subject. This study was approved by the Fujita Health University Ethics Committees.

Genotyping

Six single nucleotide polymorphisms (SNPs), five of which were the identical SNPs used in original study; SNP1 (CC-1a): rs1049437, SNP2 (CC-20): hCV1341797, SNP3 (CC-21): hCV1341817, SNP4 (CC-33): hCV3004214, SNP5 (CC-S3): rs246149 were genotyped. Another SNP (SNP6: rs7430 from dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) which is located in 3′ UTR was added as following reason. Denser SNP setting would be required especially in latter half of PPP3CC, because original positive results showed that SNPs located in latter half of PPP3CC were associated with schizophrenia.

Genomic DNA was extracted from peripheral blood of all subjects. For rapid genotyping of SNPs, polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) assays and TaqMan assays were developed.

Table 1. Primers and restriction enzymes used for genotyping

SNP	PCR Primer sequences	Product size (bp)	Annealing temperature	Restriction enzymes	Variants	Location
CC-20	5′-GGGCACAGAAAGATATACTGG-3′ 5′-GTAGAAAGTCCCACAAACTGG-3′	278	61	Xba I	T/C	Intron 1
CC-21	5′-GAAGAAGCTTCTGGCATCTGG-3′ 5′-TAGTCCCATGATCCACAGGTG-3′	312	56	Hinf I	G/A	Intron 1
CC-33	5′-GACAGAGTGAGACCCTGTCC-3′ 5′-TGACCTGCATTGACCTACATC-3′	197	56	Hpy188I	C/T	Intron 4
CC-S3	5′-GATGGAACAATAGGTCCTGG-3′ 5′-CATGCAGAGCAACACACATG-3′	266	61	Pml I	A/G	Intron 4
rs 7430	5′-AAGGGAAGAAAGCCCATTCA-3′ 5′-AAGGGAAAAACAAAACAGATGC-3′	238	56	BstNI	C/G	Untranslated

Five pairs of primers were used for typing SNP2, SNP3, SNP4, SNP5 and SNP6 for PCR-RFLP, as described in Table 1.

PCR amplification was carried out as follows. The reaction mixture was in a 6 μl volume containing a 10 ng sample DNA, 10 pmol of each primer, 2.5 mM $MgCl_2$, each dNTP at 0.2 mM and 0.3 U of AmpliTaq Gold™ (Applied Biosystems Japan Ltd.). An initial denaturation step at 95°C 9 min was followed by 40 cycles of denaturation at 95°C for 15 sec, primer annealing at each suitable temperature for 20 sec, primer extension at 72°C for 30 sec and a final extension reaction was performed at 72°C for 7 min. The PCR products were digested with each appropriate restriction enzyme (Table 1). DNA fragments were resolved by electrophoresis in a 6% acrylamide gel stained with ethidium bromide.

Concerning SNP1, the primer that was used in original study for typing SNP1 was so long that we could not type it exactly. Then we typed SNP1 with TaqMan assays (Applied Biosystems Japan Ltd.).

Statistical analysis

The presence of Hardy-Weinberg equilibrium (HWE) was examined by exact test. D′ and r^2 were calculated to evaluate pairwise linkage disequilibrium (LD). Genotype, allele and haplotype distributions between cases and controls were analyzed by COCAPHASE 2.403 (Dudbridge, 2003).

In conventional haplotypic analyses, we estimated the haplotype frequencies by expectation–maximization (EM) algorithm. We set cutoff line of haplotype frequencies less than 5% as original study defined.

An effect size was estimated by power, which was calculated using a statistical program prepared by Ohashi (Ohashi et al., 2001). The significance level for all statistical tests was 0.05.

Results

Genotype distributions in schizophrenic patients and controls are shown in Table 2. In each SNP, the genotype distributions for the two groups were in Hardy-Weinberg equilibrium. No significant differences in genotype of all SNP were observed between Japanese schizophrenic patients and controls.

Pairwise disequilibrium values of Japanese schizophrenia sample were shown in Table 3. Almost all pairwise LD and r^2 results showed the strong LD, however, CC-20 is not in strong LD with CC-33 and CC-S3 in combined

Table 2. Genotypic analysis of PPP3CC

SNP ID	Japanese sample (Frequencies in %)						
	M/M		M/m		m/m		P-value
	Patients	Controls	Patients	Controls	Patients	Controls	
CC-1a	131 (28.7)	123 (26.9)	214 (46.8)	204 (47.6)	112 (24.5)	102 (23.8)	0.88
CC-20	323 (70.7)	312 (72.7)	123 (26.9)	121 (28.2)	11 (2.4)	16 (3.7)	0.27
CC-21	222 (48.6)	220 (51.3)	195 (42.7)	179 (41.7)	40 (8.8)	30 (7.0)	0.29
CC-33	156 (34.1)	144 (33.6)	211 (46.2)	210 (49.0)	90 (19.7)	75 (17.5)	0.73
CC-S3	160 (35.0)	147 (34.3)	218 (47.7)	211 (49.2)	79 (17.3)	71 (16.6)	0.99
rs 7430	146 (31.9)	135 (31.5)	219 (47.9)	222 (51.7)	92 (20.1)	72 (16.8)	0.51

M major allele; *m* minor allele

Table 3. Pairwise disequilibrium values for PPP3CC

	Japanese sample						US sample*				Distances (bp)
	CC-1a	CC-20	CC-21	CC-33	CC-S3	rs 7430	CC-1a	CC-20	CC-21	CC-33	
CC-1a		0.08	0.36	0.58	0.55	0.25					
CC-20	0.58		0.06	0.0004	0.0003	0.08	0.72				6843
CC-21	0.89	0.87		0.46	0.49	0.38	0.98	0.78			15305
CC-33	0.85	0.04	0.91		0.84	0.34	0.89	0.39	0.67		38764
CC-S3	0.85	0.05	0.92	0.94		0.39	0.97	0.51	0.9	0.91	1683
rs 7430	0.55	0.72	0.84	0.6	0.65						37162

For each pair of markers, the standardized D′ is shown below the diagonal, and r^2 is shown above the diagonal.
* Gerber et al. (2003)

Table 4. Global haplotype association analysis of PPP3CC in Japanese Case-Control Samples

SNP ID	Global haplotype analysis (p-value[a])					
	1SNP*	2SNP	3SNP	4SNP	5SNP	6SNP
CC-1a	0.87					
		0.72				
CC-20	0.26		0.75			
		0.39		0.97		
CC-21	0.29		0.74		0.9	
		0.79		0.77		0.87
CC-33	0.72		0.75		0.63	
		0.92		0.68		
CC-S3	0.99		0.67			
		0.66				
rs 7430	0.51					

[a]p-value was calculated by COCAPHASE. * allele wise associate

Table 5. Individual haplotype analysis (5SNPs CC-1a ~ CC-S3)

5SNPs haplotype (CC-1a ~ CC-S3)	Japanese sample			US sample***		South Africa (SA) sample***		US + SA***
	Frequencies (%)		P-value[a]	Frequencies (%) (210 triads)	P-value[b]	Frequencies (%) (200 triads)	P-value[b]	P-value[b] (410 triads)
	SCZ (n = 457)	CON (n = 429)						
CTGCA	26.4	26.4	0.98	38	0.002*	43	0.07	0.0012**
CTACA	5.7	5.8	0.88	8	0.48	9	0.32	
CTATG	1.2	0.9	0.28	11	0.15	8	0.32	
ATATG	43.9	45.8	0.47	25	0.7	26	0.35	
CCACA	4.6	5.7	0.32					
CCATG	6.4	7.2	0.49					

SCZ schizophrenic. *CON* control. [a] Calculated by COCAPHASE. [b] Calculated by TRANSMITED. * In TDT results of the US samples showed a risk haplotype. ** In TDT results of the US + SA samples also showed a risk haplotype. *** Gerber et al. (2003)

sample of schizophrenics and controls. Also same trends were obtained from separated samples of schizophrenics and controls (data not shown).

Results of allele and haplotypic analysis are shown in Tables 4, 5 and 6. No significant differences in allele frequencies for all SNP were observed between Japanese schizophrenic patients and controls, including CC-21 (P = 0.75) and CC-S3 (P = 0.44) that were observed significant over-transmission in US and SA samples (P = 0.038 and 0.041, respectively).

For the conventional haplotypic analysis, like original study, we evaluated associations by two forms, global haplotypic analysis, and individual haplotypic analysis. In both analyses we constructed from two to six SNPs haplotypes from adjacent SNPs in sliding-window fashion (Table 4). In global haplotypic analysis, none of the five SNPs showed association with

Table 6. Individual haplotype analysis (6SNPs CC-1a/rs 7430)

6SNPs Haplotypes (CC-1a ~ rs 7430)	Japanese sample		
	Frequencies (%)		P-value[a]
	SCH (n = 457)	CON (n = 429)	
ATATGC	35.5	38.3	0.24
CTGCAG	24.8	25.4	0.79
ATATGG	8.5	7.5	0.39
CCATGC	5.5	6.4	0.48
CTACAG	5.6	5.7	0.94

[a] Calculated by COCAPHASE

schizophrenia. In individual haplotypic analysis of 5 SNPs for the confirmation of the contribution of individual haplotypes to global haplotypic analysis, we could not find association of major haplotypes including at-risk haplotypes (Table 5). Concerning individual haplotypic analysis of 6 SNPs (Table 6), we could not reveal association of major haplotypes to schizophrenia either.

Discussion

We have performed the first replication of association study of PPP3CC using relatively large Japanese case-control samples. For denser SNP setting, we added another SNP that is located in 3′ UTR. Although the original study showed significant associations with 2 SNPs (CC-21, CC-S3) in PPP3CC to schizophrenia, in our Japanese sample, all SNPs in PPP3CC did not show significant association with schizophrenia (Tables 2 and 4). In haplotypic analysis, we could not find significant association of major haplotypes including at-risk haplotype, either (Tables 4, 5 and 6). Our results did not confirm the previous association of PPP3CC.

This discrepancy between our study and original study might be derived from differences of the population. Japanese samples differ from US and SA samples in LD patterns (Table 3). Also, haplotypes were present in different frequencies in Japanese sample and US/SA sample (Table 5).

In addition, the magnitude of susceptibility loci from linkage analyses may be different among populations. Genome-wide linkage studies, primarily of European-Caucasian, African-American and Palauan populations, have been performed and some studies have suggested that 8p.21-22 (Pulver et al., 1995; Kendler et al., 1996; Blouin et al., 1998; Gurling et al., 2001; Straub et al., 2002), where PPP3CC is located on, is associated with susceptibility to schizophrenia. However, in genome-wide scan for linkage with Japanese schizophrenia, 8p.21-22 did not fulfill the criteria for significant or suggestive evidence for linkage (JSSLG, 2003) (Yamada et al., 2004).

To verify that our negative results might not be due to type II error, traditional power calculation based on genotype relative risk (GRR) was performed. The power was quite high and showed more than 80% power for susceptibility gene when GRR set 1.31–1.45 (Ohashi et al., 2001).

In conclusion, our results suggested that PPP3CC might not play a major role in pathophysiology of Japanese schizophrenia. However, as calcineurin is still a probable candidate gene for schizophrenia, further association analyses is required to be carried out considering locus heterogeneity among calcineurin and calcineurin-interacting molecules.

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Behavioral/Systems/Cognitive

A Variant C178T in the Regulatory Region of the Serotonin Receptor Gene *HTR3A* Modulates Neural Activation in the Human Amygdala

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Converging evidence in neurophysiological and neuroimaging studies has suggested that the limbic and prefrontal systems play important roles in emotion and cognition. These structures are activated when we see a human face, assuming that we automatically evaluate the biological significance of the stimuli. The serotonin (5-HT) system within the brain has been tied to various behaviors such as mood and anxiety and to the biology of neuropsychiatric disorders. To investigate the link between the 5-HT system and limbic/prefrontal activity, normal subjects ($n = 26$) who underwent functional magnetic resonance imaging and faced recognition tasks were genotyped for the single nucleotide polymorphism C178T in the regulatory region of the serotonin receptor type 3 gene (*HTR3A*). We found that the subjects with C/C alleles had greater activity in the amygdala and dorsal and medial prefrontal cortices than those with C/T alleles. The C/C group also showed a faster reaction time during the task than the C/T group. The temperamental predisposition of the subjects had a significant correlation with brain activity in the C/C group. The genotype effect in the right amygdala and prefrontal cortex was largest during the first run of the experiment. These results indicate that the C178T variation in the *HTR3A* has a critical influence on the amygdaloid activity and on human face processing, probably through regulation of the receptor expression. The present study may contribute to elucidating a possible link among genes, the brain, and behavior in normal populations and may help reveal the biological basis of neuropsychiatric disorders.

Key words: fMRI; face; limbic system; reaction time; personality; habituation

Introduction

Serotonin (5-HT) plays a significant role in the biological basis of human behaviors, psychiatric diseases (Meltzer, 1990), and temperamental predisposition (Reif and Lesch, 2003). Among the several subtypes of 5-HT receptors, the type 3 receptor (5-HT_3) uniquely belongs to the superfamily of ligand-gated ionotropic receptors (Turner et al., 2004) and relates with cognitive and emotive functions in humans (Olivier et al., 2000). This may be explained by the finding that the 5-HT_3 receptor is distributed in the amygdala, hippocampus, and cingulate cortex (Tecott et al., 1993; Bloom and Morales, 1998; Morales et al., 1998). Although the precise role of this receptor subtype is still elusive, several authors have suggested that the 5-HT_3 receptor inhibits memory and learning in the amygdala (Koyama et al., 2000) and hippocampus (Staubli and Xu, 1995; Bloom and Morales, 1998) through the GABAergic inhibitory mechanism (Turner et al., 2004).

The 5-HT_3 receptor gene (*HTR3A* and *HTR3B*) has been cloned for mice (Maricq et al., 1991) and humans (Miyake et al., 1995) and assigned to a single locus at human chromosome 11 (11q23.1–23.2) (Miyake et al., 1995; Weiss et al., 1995; Bruss et al., 2000). Several authors have indicated that major psychiatric disorders such as schizophrenia (Craddock and Lendon, 1999) and affective disorder (Detera-Wadleigh et al., 1998; Baysal et al., 2002) are associated with a chromosome abnormality involving distal 11q. In an *in vitro* study, a single nucleotide polymorphism (SNP), C178T, in the upstream regulatory region of the *HTR3A* gene has been found to regulate receptor expression by affecting the translation rate of HTR3A (Niesler et al., 2001). The authors suggested that the less common T allele was related to an increase of HTR3A expression compared with the more common C allele (Niesler et al., 2001). Recently, two studies demonstrated that the C178T polymorphism is relevant to the biological mechanisms of affective disorder (Nielser et al., 2001) and the personality trait of harm avoidance (HA) (Melke et al., 2003). Therefore, it is particularly important to investigate the functional relevance of this SNP and brain activity in normal human subjects.

For this purpose, 26 subjects who underwent functional mag-

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Table 1. Demographic characteristics, personality/depression score, and 5-HTTLPR genotype of the C/C and C/T groups

C178T genotype	C/C	C/T
Number of subjects	15	11
Sex (male/female)	6/9	7/4
Mean age	23.4 (3.8)	21.6 (2.1)
Education (years)	15.8 (1.5)	15.6 (1.2)
TCI		
HA	12.0 (4.8)	11.1 (4.3)
NS	9.8 (4.1)	9.9 (4.1)
RD	9.5 (1.9)	9.9 (2.8)
Persistence	2.4 (1.1)	2.6 (1.2)
BDI	3.9 (4.1)	4.0 (4.6)
5-HTTLPR	s/s, 10; s/l, 5	s/s, 4; s/l, 5; l/l, 2

Values are the mean and SD of each group. The ethnicity of the subjects is Japanese. 5-HTTLPR, Number of subjects in each genotype.

netic resonance imaging (fMRI) and a face recognition task were genotyped, and the blood oxygen level-dependent (BOLD) signal changes were compared between groups with different genotypes. During the task, pictures of unfamiliar faces with neutral expressions or pictures of houses were presented. The subjects were instructed to determine whether the picture was of a face or house. Face recognition was used because this task has been shown to predominantly activate the amygdala in normal subjects (for review, see Zald, 2003). The subject's personality trait as measured by the Temperament and Character Inventory (TCI) scale (Cloninger, 1987) was examined in relation to the brain activity and genotype. We predicted that the signals in the amygdala would be reduced in subjects with a T allele, because this allele is associated with increased receptor expression (Nielser et al., 2001) and subsequently involves the GABAergic inhibitory mechanism (Turner et al., 2004). Furthermore, responses in the human amygdala have been shown to change across experimental runs (Breiter et al., 1996). In the present study, the experimental run was repeated three times to investigate the genetic modulation of habituation in amygdaloid activation.

Materials and Methods

Subjects. Twenty-six right-handed healthy subjects participated in the experiment after providing written informed consent. Demographic characteristics of the subjects are summarized in Table 1. The study was approved by the ethics committee at the National Institute for Physiological Sciences and Fujita Health University and by the Institutional Review Board at the Nagoya University Graduate School of Environmental Studies. The subjects completed the Japanese versions of the Beck Depression Inventory (BDI) (Beck and Steer, 1993) and the TCI (125 items) (Cloninger, 1987)

Genotyping of C178T: PCR-restriction fragment length polymorphism. A blood sample was collected from each subject, and genomic DNA was extracted using standard methods. The primer pairs were 5′-TTTCCTCCCGCCTGAAAC-3′ and 5′-AAGTCCTGCTGCTTCCCG-3′. DNA amplification was performed using an iCycler (Bio-Rad Laboratories Japan, Tokyo, Japan). The reaction mixture was in a 6 μl volume containing 10 ng of sample DNA, a 0.25 mM concentration of each primer, and 200 mM each of deoxy NTP, 1× PCR Gold buffer, 2 mM $MgCl_2$, 5% DMSO, and 0.15 U of AmpliTaq Gold (Applied Biosystems Japan, Tokyo, Japan). Initial denaturation at 95°C for 9 min was followed by 45 cycles of denaturation at 95°C for 15 s, primer annealing at 57°C for 20 s, and primer extension at 72°C for 30 s, with a final extension reaction at 72°C for 7 min. Then, 6 μl of the PCR product was digested with 1 U of restriction enzyme, Hpy188III, according to the manufacturer's recommendations. DNA fragments were resolved by electrophoresis in a 6% acrylamide gel stained with ethidium bromide. In addition to the C178T polymorphism of HTR3A, the subjects were genotyped for the 5-HTTLPR polymorphism according to the method described by Lesch et al. (1996).

Experimental procedure. Digitized grayscale pictures of 48 faces with neutral expressions taken from posers (24 males and 24 females) and pictures of 20 houses created using computer graphics software (Aska-Pro; Logic, Kanazawa, Japan) served as the stimuli (Iidaka et al., 2001). These pictures were divided into two sets of stimuli, which were assigned randomly to each subject. For each face picture, an inverted version and inverted-blurred version were created with equal luminance using commercial photographic software. In each run, 24 upright faces, 24 inverted faces, 24 inverted-blurred faces, 10 houses, and 24 null events with fixation were presented randomly. Each run was repeated three times with the same set of stimuli intermixed randomly. Although in the original experiment the inverted and inverted-blurred face conditions were included, the results for these conditions are not reported here. Hereafter, we will refer to the "upright face" condition as the "face" condition. Each subject saw these stimuli successively, one at a time, for 500 ms with an interstimulus interval of 4500 ms. During the interval, a fixation point was shown. In the fMRI experiment, the stimuli were projected onto a transparent screen hanging on the bore of a magnet 75 cm from the subject's eyes. The subjects viewed the stimuli through a tilted mirror attached to the head coil of the scanner. All of the stimuli subtended a visual angle of ~5.5 × 7°. The subject was asked to judge whether the presented stimulus was a face or a house and press a corresponding button with his or her right hand. The reaction time (RT) for the task during the fMRI experiment was compared between genotypes using one-way ANOVA and is plotted in Figure 1.

fMRI data acquisition and analysis. Functional images of the entire brain were acquired in an axial orientation using a 3 tesla Siemens (Erlangen, Germany) Allegra MRI scanner equipped with single-shot echo planar image [EPI; repetition time (TR), 2.3 s; echo time (TE), 30 ms; flip angle, 80°; 64 × 64 matrix; 36 slices; voxel size, 3 × 3 × 3 mm] sensitive to BOLD contrast (Ogawa et al., 1992). After discarding the first 6 images, the remaining 234 successive images in each run were subjected to analysis. A high-resolution anatomical T1-weighted image was also acquired (MPRAGE; TR, 2.5 s; TE, 4.38 ms; flip angle, 8°; 256 × 256 matrix; 192 slices; voxel size, 0.75 × 0.75 × 1 mm) for each subject. The fMRI experiment was controlled using Presentation software (Neurobehavioral Systems, Albany, CA).

Data were analyzed by SPM99 (Wellcome Department of Imaging Neuroscience, London, UK). First, all volumes were realigned spatially to the final volume, and the signal in each slice was realigned temporally to that obtained in the middle slice using a sinc interpolation. The resliced volumes were normalized to the MNI (Montreal Neurological Institute) space (Evans et al., 1993) using a transformation matrix obtained from the normalization process of the mean EPI of each individual subject to the EPI template. The normalized images were spatially smoothed with an 8 mm Gaussian kernel.

After preprocessing, statistical analysis of each individual subject was conducted using the general linear model (Friston et al., 1995). At the first level, each single event was modeled as a hemodynamic response function and its temporal derivative. Low-pass and high-pass frequency filters were applied to the time-series data. For each subject, motion parameters were included as regressors of no interest to take into account the effects of head motion not removed at the realignment stage. The images were scaled to a grand mean of 100 over all voxels and scans within each session. In the subtraction analysis, five conditions (correct responses for the four experimental conditions and incorrect responses) were modeled separately. Parameter estimates for each condition and for the difference between the conditions were calculated from the least-mean-square fit of the model to the time-series data. Our particular interest was in the images of parameter estimates representing the difference in event-related activity between the face condition and house condition.

At the second-level (random-effects) analysis, we compared the brain activity between genotypes by entering contrast images of each individual subject containing parameter estimates representing activation for the face minus house condition into a two-sample t test. The statistical threshold of the analysis was set at $p = 0.001$, uncorrected for multiple

comparisons for height; clusters were >5 voxels. This height threshold was chosen because the difference in amygdaloid activity between the genotypes was predicted as noted in the Introduction. Region names, coordinates, and Z values for the difference between the genotypes are tabulated in Table 2. Amygdaloid and prefrontal regions with a significant group difference are shown in Figures 2 and 3, respectively. Signals extracted from the four regions with a significant group difference are plotted in Figure 4. These signals were extracted from the spherical regions of interest that were drawn on each region with a radius of 8 mm using MarsBaR software (Brett et al., 2002). As supplementary analyses, the subject's age or sex (male or female as a categorical variable) was included in the analysis of covariance to exclude the effect of these factors from the results for group differences.

Table 2. Brain regions with significant difference in activation between the genotype

	Region name	R/L	Voxel size	Z value	Coordinate
C/C > C/T					
	Middle frontal gyrus (BA9)	R	17	3.82	44, 30, 32
	Intraparietal sulcus (BA7)	L	11	3.58	−26, −40, 52
	SMA (BA6)	R	14	3.50	4, −22, 48
	Amygdala	R	9	3.48	22, −4, −30
	Superior frontal gyrus (BA10)	R	16	3.45	20, 58, 24
C/T > C/T					
	Fusiform gyrus (BA37)	R	27	3.42	38, −62, −30

R, Right hemisphere; L, left hemisphere; SMA, supplementary motor area.

To examine the relationship between the temperamental predisposition, genotype, and brain responses, multiple regression analyses were conducted using the score of the TCI subscale, the signal change in the amygdala and prefrontal areas, and the genotype as variables (Hariri et al., 2005). Significance of the interaction effect of genotype and BOLD signal on the TCI subscale was tested at the $p = 0.05$ level. The correlations between the right amygdala activity and HA score, between the right superior frontal gyrus activity and novelty-seeking (NS) score, and between the medial prefrontal activity and reward dependence (RD) score are plotted in Figure 6.

To test whether the temporal pattern of activation differs between the genotypes, the contrast image of the face minus house condition was created separately for each run. Separate ANOVAs with the RT as a covariate were conducted for the first, second, and third runs at the threshold of $p = 0.001$, uncorrected, and $k = 10$ voxels. The analysis was restricted to the right amygdala and prefrontal cortices where significant group difference was observed. Signal changes during each run were extracted from the right amygdala and superior frontal gyrus and are shown in Figure 7. The results shown in Tables 1 and 2 and in Figures 1–6 are from the analyses collapsing the three experimental runs.

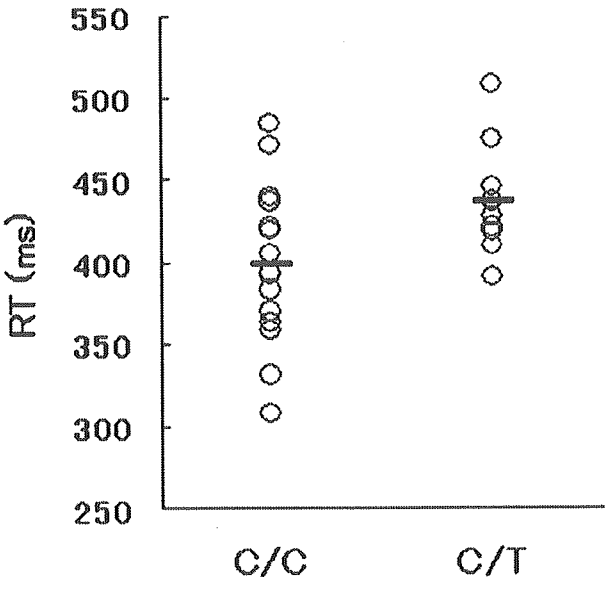

Figure 1. Reaction time (RT) during the fMRI experiment in each individual subject and group; the mean value (horizontal bar) is shown. The C/C group had a significantly faster mean RT than the C/T group.

Results

Genomic data

For the C178T polymorphism, 15 subjects had C/C alleles and 11 subjects had C/T alleles (Table 1). The genotypes were distributed according to the Hardy-Weinberg equilibrium. These two groups did not differ significantly in terms of mean age (Kruskal–Wallis test; $p = 0.24$), sex ($\chi^2 = 1.41$; $p = 0.23$), or years of education ($F = 0.02$; $p = 0.88$). The proportion of each of the 5-HTTLPR variants did not differ significantly between the C/C and C/T groups ($\chi^2 = 4.05$; $p = 0.13$).

Behavioral data

The mean RT and accuracy for the face recognition task during the fMRI experiment were 413 ± 46 ms and 97 ± 5%, respectively. One-way ANOVA showed that the main effect of the genotype on the mean RT was significant ($F = 5.26$; $p = 0.03$). The subjects with C/C alleles had faster RTs than those with C/T alleles (398 ± 48 vs 437 ± 31 ms), as plotted in Figure 1. There was no significant group difference in accuracy during the fMRI experiment ($F = 1.83$; $p = 0.18$). Neither the mean of the BDI score nor that of the four TCI subscales differed significantly between the genotypes (Table 1).

Neuroimaging data

There were several brain regions where the signal change in response to face stimuli compared with house stimuli was significantly greater for the subjects with C/C alleles than for those with C/T alleles. These regions were located in the right amygdala (Fig. 2), right prefrontal cortex (Fig. 3), supplementary motor area, and left intraparietal sulcus (Table 2). A cluster in the right fusiform gyrus showed greater activation in the C/T group than in the C/C group. These six regions survived small volume correction for height within an 8 mm sphere at the $p = 0.05$ level. The regions also remained significant ($p = 0.001$, uncorrected) when the subject's age or sex was included in the analysis as a nuisance covariate. The degree of activation in the peak voxel listed in

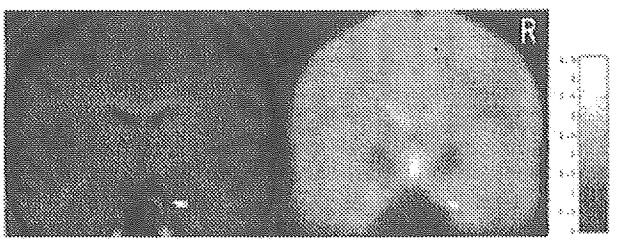

Figure 2. The right amygdala with significant group differences in activation measured by fMRI is superimposed on a high-resolution T1-weighted image of a single subject (left) and on a mean EPI of 26 subjects (right). Coronal images are shown at $y = -4$ mm. In this region, the signal was greater for the C/C group than for the C/T group. For coordinates, voxel size, and Z values, see Table 2. R, Right.

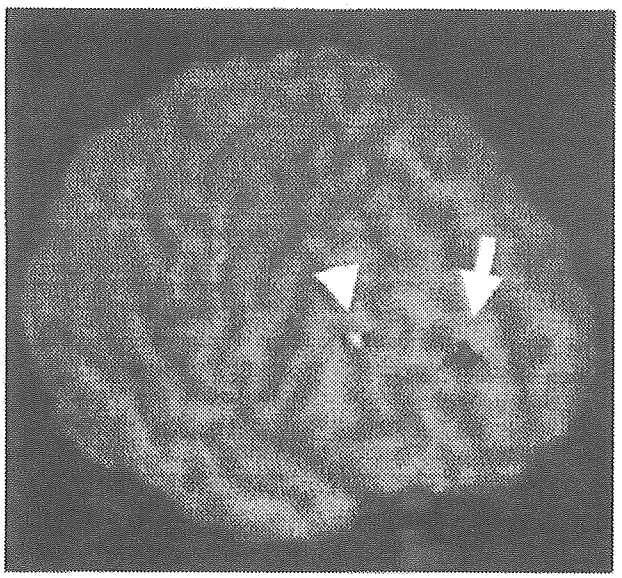

Figure 3. The areas on the right superior frontal gyrus (BA10; arrow) and middle frontal gyrus (BA9; arrowhead) where the signal was greater for the C/C group than for the C/T group are rendered on the surface of a single subject's brain. For coordinates, voxel size, and *Z* values, see Table 2.

Table 2 did not differ between male and female subjects in either the C/C or C/T group at a lenient threshold ($p = 0.05$, uncorrected). The signals extracted from four regions in each individual subject are plotted in Figure 4. When two subjects with the l/l genotype of the 5-HTTLPR variant were excluded from the analysis, the six regions listed in Table 2 showed significant difference in activation between the C178T genotypes ($p = 0.001$, uncorrected). Therefore, the effects of C178T variants on activity in the amygdala and other regions do not simply reflect the influence of the 5-HTTLPR genotype.

In addition to these regions, we were particularly interested in the medial prefrontal cortex [mPFC; Brodmann's area 9 (BA9)] (Fig. 5, $x, y, z = -10, 52, 32$), which survived the height threshold ($z = 3.14$; $p = 0.001$, uncorrected) but not the extent threshold (k = 3 voxels), because this region has reciprocal connection with the amygdala (Ongur and Price, 2000). As shown in the right side of Figure 5, the mPFC activity positively correlated with the amygdaloid activity across all 26 subjects, suggesting that these two regions may have a functional relationship during the face recognition task. To examine whether the functional coupling of the amygdala and mPFC would be modulated by genotype or experimental run, the correlation coefficient was computed separately for each run and genotype. A positive correlation was significant only during the first ($r = 0.58$; $p < 0.05$) and second ($r = 0.58$; $p < 0.05$) runs in the C/C group.

The functional relationship between the temperamental predisposition as measured by the TCI subscale and BOLD signal is modulated by the C178T genotype as shown in Figure 6. The right amygdala activity is significantly and negatively correlated with the HA score in the C/C group ($r = -0.69$; $p = 0.004$) but not in the C/T group ($r = 0.21$; $p = 0.52$). Activity in the right superior frontal gyrus (BA10) had a significant positive correlation with the NS score in the C/C group ($r = 0.51$; $p = 0.03$) but not in the C/T group ($r = -0.41$; $p = 0.19$). Finally, the RD score significantly and negatively correlated with mPFC (BA9) activity in the C/C group ($r = -0.57$; $p = 0.02$) but not in the C/T group

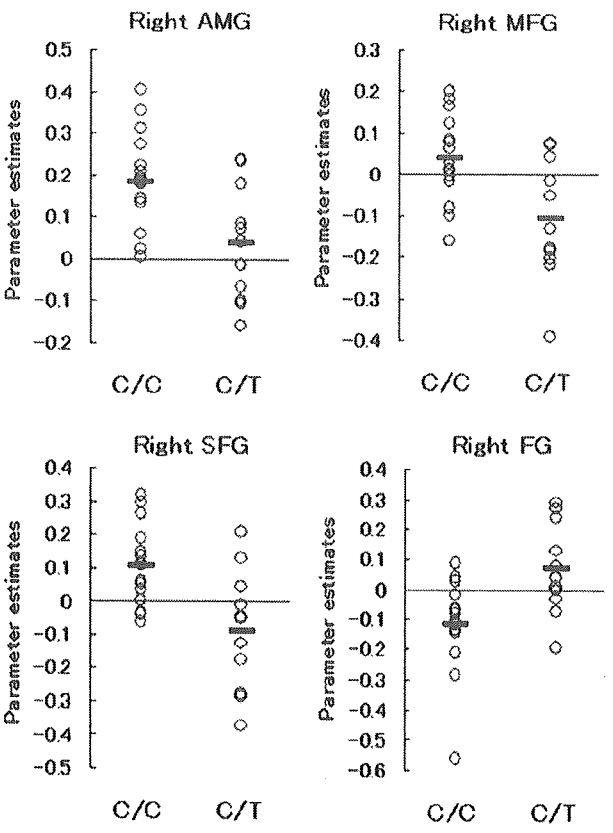

Figure 4. Signal changes in response to face stimuli compared with house stimuli in each subject extracted from spherical ROIs ($r = 8$ mm) at the right amygdala (AMG), middle frontal gyrus (MFG; BA9), superior frontal gyrus (SFG; BA10), and fusiform gyrus (FG; BA37) are shown. The horizontal bar indicates the group mean.

($r = 0.51$; $p = 0.37$). In these cases, multiple regression analyses showed a significant genotype-by-BOLD interaction effect on the HA ($p < 0.05$), NS ($p < 0.05$), and RD ($p < 0.01$) scores.

Temporal pattern of activation

The right amygdala ($x, y, z = 26, -4, -28$) and superior frontal gyrus (BA10; $x, y, z = 18, 50, 24$) showed a significant difference in activation between the genotypes only during the first run when the difference in the RT was taken into account. As shown in Figure 7, the subjects with C/C alleles had higher signals in these regions than those with C/T alleles, particularly during the first run. We conducted two-way ANOVA with a Greenhouse-Geisser correction for the run (within subjects) and genotype (between subjects) as factors on signal and RT data. There was a significant interaction effect of the run and genotype on the BOLD signal (amygdala: $F = 3.85$, $p < 0.05$; superior frontal gyrus: $F = 4.39$, $p < 0.05$). A *post hoc t* test showed that the genotype effect was significant during the first ($p < 0.01$) and third ($p < 0.05$) runs for the amygdala and during the first run ($p < 0.01$) for the prefrontal cortex. The interaction effect of the run and genotype on the RT was not significant.

Discussion

Combined neurophysiological and genetic studies have been suggested as an important research strategy in the field of neuroscience (Begleiter et al., 1984; Porjesz et al., 2002; Hariri and Weinberger, 2003). The study by Hariri et al. (2002) showed an