association between amygdaloid activity and the 5-HT transporter gene had an impact on research into mood disorders, because 5-HT is known to be related to depression and stressful life events (Caspi et al., 2003). The present study, in a similar vein as their report, investigated whether the SNP, C178T, in the upstream regulatory region of the *HTR3A* gene would modulate brain activity in the limbic areas during a face recognition task. There were significant differences in the BOLD signals measured in the right amygdala and prefrontal cortices between the subjects with C/C alleles and those with C/T alleles. These effects were not simply a reflection of the 5-HTTLPR variants of the subjects. The two groups of subjects also differed in the RT obtained during the fMRI experiment. In addition, the genetic effect on the relationship between the temperament and BOLD signal was significant. In the right amygdala and prefrontal regions, the temporal pattern of activation across runs was modulated by genotype. The present findings indicate a possible role of this genetic polymorphism in face processing through neural responses in the amygdala and prefrontal system, which underlie complex human behaviors.

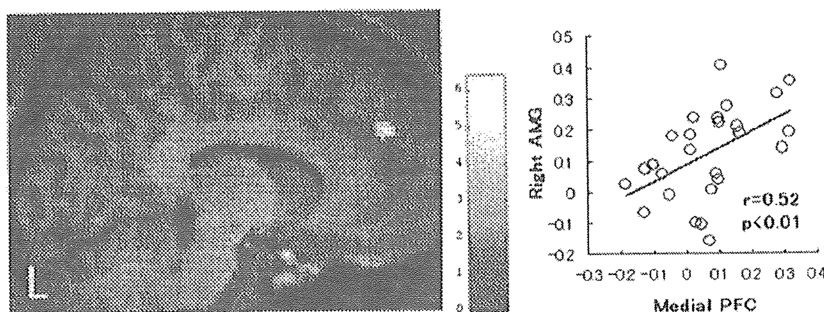


**Figure 5.** Left, mPFC (BA9; $x, y, z = -10, 52, 32$) where the signal was significantly greater for the C/C group than for the C/T group. A cluster is superimposed on a high-resolution T1-weighted image ($x = -10$ mm). The height threshold is set at $p = 0.005$, uncorrected for multiple comparisons for the purpose of presentation. Right, Significant positive correlation between right amygdaloid activity and medial prefrontal activity. A regression line, a correlation coefficient, and a $p$ value are also shown. AMG, Amygdala; PFC, prefrontal cortex.

The group fMRI analysis clearly showed that the degree of activation in the right amygdala differed between the subjects with C/C alleles and those with C/T alleles. Changes in the BOLD signal imply changes in the local concentration of oxy-/deoxy-hemoglobin (Ogawa et al., 1992), but not the direct synaptic activity associated with the release and the receptor binding of particular neurotransmitters. However, several findings indicate that the genetic polymorphism of the 5-$HT_3$ system may alter hemodynamic responses in specific regions of the human brain during cognitive tasks. First, the 5-$HT_3$ receptor is preferentially expressed in the amygdala and the cingulate gyrus in animals and humans (Tecott et al., 1993; Bloom and Morales, 1998; Morales et al., 1998). Second, in an *in vitro* study, the T allele of the C178T polymorphism has been suggested to regulate receptor expression by enhancing the translation rate of the downstream HTR3A (Niesler et al., 2001). Third, a positron emission tomographic study showed that administration of the 5-$HT_3$ receptor antagonist altered the amygdaloid blood flow in human subjects (Berman et al., 2002). Finally, *in vitro* studies found that the specific 5-$HT_3$ receptor agonist facilitates inhibitory GABA release in the amygdala (Koyama et al., 2000), hippocampus (Turner et al., 2004), and other areas (Bloom and Morales, 1998). These results may suggest that the right amygdaloid function in the subjects with a T allele is inhibited by enhanced receptor expression and GABAergic neurotransmission.

In addition to the limbic regions, two dorsolateral prefrontal cortices had greater activation in the C/C group than in the C/T group. These areas are particularly involved in the processes of working memory and episodic encoding of nonverbal materials such as human faces (Kelley et al., 1998). Higher-order operations on facial information in the prefrontal cortex may be conducted extensively in the subjects with C/C alleles. In contrast, activity in the right fusiform gyrus, a region that is sensitive to the human face (Allison et al., 1994), was greater in the C/T group than in the C/C group. This may be as a result of the subjects with the T allele relying more heavily on the visual cortical pathway to process the stimuli than the other subjects, who used the amygdala-mediated subcortical route. Strong right-lateralization of activation would be attributable to that the right hemisphere is more specialized for face processing in human subjects than the left hemisphere (Sergent and Bindra, 1981).

A novel finding of the present study is that the relationship between the TCI score and brain response was modulated by genotype, although the mean TCI score did not differ between the two groups. A significant negative correlation between HA and amygdaloid activity may imply that enhanced 5-HT function in those with a high HA score reduced neural response in the limbic region in the C/C group (Del-Ben et al., 2005). A positive correlation between activity in the superior frontal gyrus and the NS in the C/C group may relate to that the subjects with high extraversion scores having greater prefrontal activation during working memory tasks than those with low extraversion scores (Kumari et al., 2004). Finally, the correlation between the medial prefrontal activity and RD may be underpinned by the evidence that the medial prefrontal–limbic circuit subserves reward processing in experimental animals (Wise, 2000).

The RT during the fMRI experiment differed between the genotypes, indicating that the subjects with the T allele do not process face stimuli as fast as those who are homozygous for the C allele. Amygdaloid function has been implicated in conditioned associative learning (Ono et al., 1995) and in the processing of stimuli with biological significance in multiple sensory domains (Zald, 2003). The amygdala is a part of the subcortical information pathway that bypasses the primary visual cortex to respond rapidly to salient stimuli (Morris et al., 1999; Adolphs, 2002). The present results may suggest, in accordance with this model, that a shorter mean RT in the C/C group is a reflection of activation in the amygdala-mediated subcortical pathway. The result that the C/T group, which used the right fusiform gyrus during the task, responded more slowly than the C/C group fits with this hypothesis.

Neuroimaging studies in normal subjects have shown that the amygdaloid and prefrontal responses to face stimuli are largest during the initial phase of the experiment (Breiter et al., 1996; Wright et al., 2001). In contrast, an fMRI study showed that the peak of the amygdaloid response in depressed subjects occurred later in the time course than that in control subjects (Siegle et al., 2002). Therefore, we hypothesized that the genotype effect in the amygdala would be found predominantly during the first run of the experiment. As shown in Figure 7, the difference in activation between the groups was largest during the first run in the right amygdala and superior frontal gyrus. The C/C group showed a

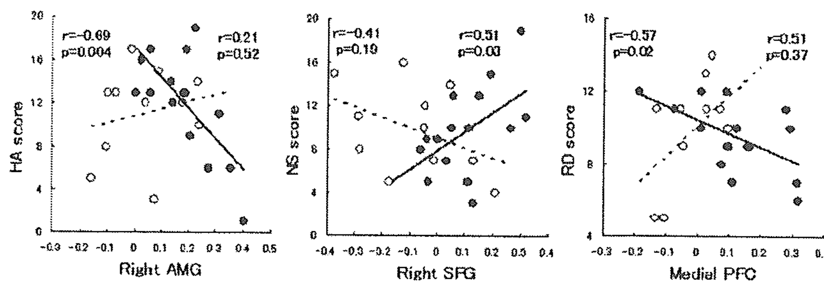


**Figure 6.** Correlations between the TCI subscale score and the brain activity are plotted separately for each genotype group. Left, Harm avoidance (HA) score and right amygdaloid activity. AMG, Amygdala. Middle, Novelty-seeking (NS) score and activity in the right superior frontal gyrus (SFG; BA10). Right, Reward dependence (RD) score and medial prefrontal cortex (PFC; BA9) activity. Filled circles indicate subjects with C/C alleles, and open circles indicate subjects with C/T alleles. The regression lines (C/C group, solid line; C/T group, dotted line), correlation coefficients, and $p$ values are also shown.

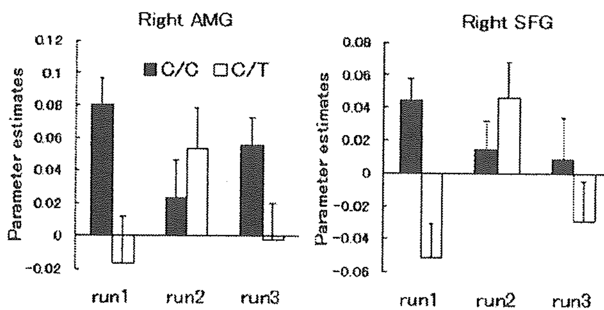


**Figure 7.** The mean and SE of the right amygdala (AMG; $x, y, z$ = 24, −4, −30) and prefrontal [superior frontal gyrus (SFG); BA10; $x, y, z$ = 18, 52, 24] responses to face stimuli in each group of subjects during the first (run1), second (run2), and third (run3) runs of the fMRI experiment are shown. ■, C/C group; □, C/T group.

typical temporal pattern of activation, whereas in the C/T group, the peak was observed in the second run. Reduced activation and the differential habituation pattern would be related to disturbance in stress hormone response through the 5-HT system (Van Praag, 2004).

Another significant finding was that the mPFC activation was greater in the C/C group than in the C/T group and positively correlated with the amygdaloid activation. Functional coupling of mPFC and the amygdala was significant during the first and the second runs in the subjects with C/C alleles. The mPFC plays a role in reward processing and goal-directed behavior in animals (Wise, 2000) and in humans (Rogers et al., 2004) and exerts significant control over the emotional–cognitive aspect of behavior (Vertes, 2004). Neuroanatomical studies in animals have shown that the mPFC has reciprocal connections with several nuclei of the amygdala (Ongur and Price, 2000), thalamus, and brainstem (Vertes, 2004) and relates with hormonal response to stress through the hypothalamic–pituitary–adrenal axis (Crane et al., 2003).

The present results have clinical implications in neuropsychiatry, because the C178T variant was more frequently found in bipolar affective patients than in a control group (Nielser et al., 2001). Differential BOLD signals and blood flow activation in the region of the amygdala, compared with normal subjects, have been observed in patients with mood disorders (Drevets, 2003) and anxiety disorders (Anand and Shekhar, 2003). An increased stress hormone level found in patients with depression and an anxiety disorder (Van Praag, 2004) is a biological marker of susceptibility to socio-psychological stress. Therefore, it is suggested that the subjects with the T allele and an increased $5\text{-HT}_3$ receptor expression (Nielser et al., 2001) may have an elevated hormonal level because the $5\text{-HT}_3$ receptor is related to ACTH secretion in response to acute stress (Bhatnagar et al., 2004).

There are several caveats in the present study. First, the number of subjects is small and differed between the groups. In the future, a study with a larger number of subjects should be conducted. Second, the amygdala activity might have been limited because we used neutral faces, which elicit less activation in this region than faces expressing emotion, as stimuli. Third, the fact that there were no significant differences in the mean TCI subscale scores or BDI score between the genotypes may have obscured the functional relevance of the amygdala to personality and emotion. However, a recent study of HTR3A polymorphism and personality traits also showed no differences in the HA scores between the C/C and C/T groups (Melke et al., 2003). Neurophysiological responses as measured by fMRI would be more sensitive than questionnaires such as the BDI and TCI for detecting group differences. Neural activation in the amygdala should be treated as an endophenotype underlying both normal and pathological brain functions (Almasy and Blangero, 2001).

In conclusion, the present study, to the best of our knowledge, revealed for the first time the significant effect of HTR3A polymorphism on the amygdala and prefrontal activity as measured by fMRI. In addition, it showed that the difference in behavioral performance during a face recognition task was related to the genetic variation, indicating a close coupling of genes, behavior, and neurophysiological responses. The present findings may have clinical implications in predicting an individual's vulnerability to socio-psychological stressors before the onset of neuropsychiatric disorders. However, the causal relationship between genotype and brain activation is not fully elucidated in the present study. Therefore, additional investigation involving *in vitro* as well as *in vivo* experiments using animals, normal volunteers, and clinical populations are needed in the future.

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# Positive Association of the Serotonin 5-HT$_7$ Receptor Gene with Schizophrenia in a Japanese Population

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Several lines of evidence suggest that abnormalities in the serotonin system may be related to the pathophysiology of schizophrenia. The 5-HT$_7$ receptor is considered to be a possible schizophrenia-susceptibility factor, based on findings from binding, animal, postmortem, and genomewide linkage studies. In this study, we conducted linkage disequilibrium (LD) mapping of the human 5-HT$_7$ receptor gene (HTR7) and selected four 'haplotype-tagging (ht) SNPs'. Using these four htSNPs, we then conducted an LD case-control association analysis in 383 Japanese schizophrenia patients and 351 controls. Two htSNPs (SNP2 and SNP5) and haplotypes were found to be associated with schizophrenia. A promoter SNP (SNP2) was further assessed in a dual-luciferase reporter assay, but it was not found to have any functional relevance. Although we failed to find an actual susceptibility variant that could modify the function of HTR7, our results support the supposition that HTR7 is a susceptibility gene for schizophrenia in this ethnic group.
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**Keywords:** schizophrenia; 5-HT$_7$ receptors; linkage disequilibrium; htSNP; haplotype

## INTRODUCTION

The human 5-hydroxytryptamine 7 (5-HT$_7$) serotonin receptor is a G-protein-coupled receptor (Hoyer *et al*, 2002). These receptors are expressed in discrete areas of the brain as well as in both vascular and gastrointestinal smooth muscle (Bard *et al*, 1993; Lovenberg *et al*, 1993), and the recent availability of selective antagonists and knockout mice strains has increased our knowledge about their functional roles. The 5-HT$_7$ receptors are now known to have an important role in modulating hippocampal neuronal functions such as learning and memory, disturbances in which are thought to be fundamental in schizophrenia (Hedlund and Sutcliffe, 2004; Thomas and Hagan, 2004). 5-HT$_7$ receptor knockout mice have also exhibited a specific impairment in contextual fear conditioning, which is associated with hippocampus-dependent learning, and reduced ability for long-term potentiation in the CA1 region of the hippocampus (Roberts *et al*, 2004).

Other *in vivo* and *in vitro* studies suggest that 5-HT$_7$ receptors can mediate the effects of 5-HT on hippocampus glucocorticoid receptor expression (Weaver *et al*, 2001; Laplante *et al*, 2002; Beique *et al*, 2004). Glucocorticoid has been tentatively associated with neurotoxicity, and considering the neurodevelopmental hypothesis of schizophrenia might have some role in this disease (Cotter and Pariante, 2002).

Neuropsychopharmacologically, 5-HT$_7$ receptors show high affinity for a number of antidepressants and typical and atypical antipsychotics. For example, clozapine, which is a classical atypical antipsychotic drug and which has been shown to be effective in treating schizophrenia (Safferman *et al*, 1991; Kane *et al*, 2001), exhibits moderate potency as a 5-HT$_7$ receptor antagonist. This finding suggests that an interaction between and/or overactivity of receptor systems may be involved in the pathophysiology of schizophrenia, although the pharmacological basis of the unique actions of clozapine is not well understood. Other lines of evidence also support the association of 5-HT$_7$ receptors to schizophrenia; A postmortem study showed decreased expression of 5-HT$_7$ receptors in the dorsolateral prefrontal cortex of schizophrenics (East *et al*, 2002). Furthermore, genomewide linkage studies of schizophrenia have shown a linkage in 10q22 (Mowry *et al*, 2000; Fallin *et al*, 2003) (OMIM: SCZD11,%608078) close to the location of the human 5-HT$_7$ receptor gene (HTR7: 10q21–24).

Here, we evaluate whether HTR7 is associated with schizophrenia in a sample including 383 unrelated Japanese schizophrenia patients and 351 unrelated controls. A common hypothesis about allelic architecture proposes that

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the greatest genetic risk for common, complex diseases including schizophrenia is from disease loci that have one common variant (common disease–common variants hypothesis: CD–CV hypothesis) (Chakravarti, 1999). In the light of this CD–CV hypothesis, association analysis using linkage disequilibrium (LD) mapping would seem to be for a reasonable approach to narrow down the number of potential susceptibility genes or variants for schizophrenia. We therefore performed (1) LD mapping in HTR7 and selection of 'haplotype-tagging (ht) SNPs', (2) an association analysis using 'htSNPs', and (3) a systematic mutation search to detect actual susceptibility variants capable of modifying the function of HTR7.

## MATERIALS AND METHODS

### Subjects

A total of 383 patients with schizophrenia (200 male and 183 female; mean age ± standard deviation (SD), 42.6 ± 14.5 years) participated in the present study. The subjects for the mutation search were 48 schizophrenic patients who were also among the 383 patients in the association analysis (27 male and 21 female; mean age ± SD, 39.5 ± 14.7 years). A total of 351 healthy volunteers (193 male and 158 female; mean age ± SD, 33.5 ± 13.1 years) were recruited as control subjects. The subjects for initial 'LD mapping' were 96 controls who were also subjects in the association analysis.

The characterization details and psychiatric assessment of these subjects were identical to those published elsewhere (Suzuki *et al*, 2003; Ikeda *et al*, 2005). The patients were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and review of medical records. All healthy controls were also psychiatrically screened based on unstructured interviews. All subjects were unrelated to each other and ethnically Japanese, and were individually matched for gender and geographical origin.

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 Graduate School of Medicine.

### SNP Inclusion for LD Mapping

We included all SNPs from the dbSNP database (URL; http://www.ncbi.nlm.nih.gov/SNP/) and Celera Discovery System database (URL; http://www.celeradiscoverysystem.com/) for LD mapping (Table 1 and Figure 1).

### Selection of 'htSNPs'

'LD blocks' were first determined using criteria based on 95% confidential bounds on $D'$ values (Gabriel *et al*, 2002), with Haploview version 2.05 software (Barrett *et al*, 2004). 'htSNPs' were then selected within each 'LD block' for 90% haplotype coverage using SNPtagger software (Ke and Cardon, 2003). This strategy for association analyses after initial LD mapping and 'htSNPs' selection is considered to be reasonable on the basis of descriptions given in other papers (Kamatani *et al*, 2004; van den Oord and Neale, 2004).

### SNP Genotyping

Genomic DNA was extracted from peripheral blood of all subjects. For rapid genotyping of SNPs, we used TaqMan assays (SNP1, SNP3, SNP4, SNP5, and SNP7), polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assays (SNP6), primer extension with denaturing high performance liquid chromatography (dHPLC) (SNP2) and direct sequencing (SNP8) (Table 1). TaqMan probes and Universal PCR Master Mix were from Applied Biosystems (CA). A 5-μl total reaction volume was used and, after PCR, the allelic specific fluorescence was measured on an ABI PRISM 7900 Sequence Detector System (Applied Biosystems, CA). Methods for RFLP assays, primer extension, and direct sequencing were described in greater detail previously (Suzuki *et al*, 2003).

### Dual-Luciferase Reporter Assay

Fragments of 262 bp including SNP2 were PCR amplified. Genomic DNAs with identified genotypes were used as templates, and PCR products of both genotypes were cloned into pGL3-promoter Vector (Promega, WI). These Vectors with both alleles and the *Renilla* luciferase vector, phRL-TK vector, were transiently transfected into Chinese hamster ovary (CHO) cells using Lipofectamine 2000™ (Invitrogen, CA). After 48 h, cell extracts were prepared and assayed for firefly luciferase activity ($LA_F$) and *Renilla* luciferase activity ($LA_R$) as described by the manufacturer (PikkaGene Dual SeaPansy™ Luminescence Kit, Toyo Ink, Japan) on a Fluoroskan Ascent FL (Thermo Labsystems, Finland). All experiments were repeated at least three times. To correct for transfection efficiency, the relative luciferase activity (RLA) was calculated as: $RLA = LA_F/LA_R$. The RLA of mutants (RLAmt) was shown by % ratio of the RLA of wildtype (RLAwt). Means and standard errors were calculated and an unpaired two-tailed t-test was performed using the software package SPSS 10.0J (SPSS Japan Inc.,

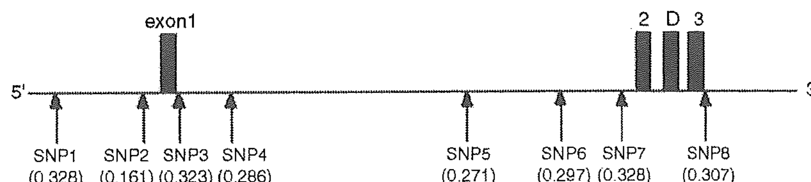


**Figure 1** Genomic structure of HTR7 with SNPs used in LD mapping. Exon D is an alternative splice exon. Numbers in parentheses represent minor allele frequencies (MAFs) of 96 controls.

Table 1 Summary of SNPs in HTR7

| SNP | SNP ID | Distance to next SNP (bp) | Methods | Primer sequences for PCR | | Primer sequence for primer extension | Restriction enzyme |
|---|---|---|---|---|---|---|---|
| | | | | Forward | Reverse | | |
| SNP1 (C>T) | rs10509613 (C206914) | — | TaqMan | | | | |
| SNP2 (G>A) | rs3808932 | 34915 | Primer extension | CTTCCCATAAGCGGTCAAAA | GTCCTTGCCGCATGATAGAT | GAGACGTTTTTTGGTTGTT | |
| SNP3 (T>C) | rs7919103 (C368389) | 3019 | TaqMan | | | | |
| SNP4 (G>A) | rs2185706 (C1175684 1) | 12040 | TaqMan | | | | |
| SNP5 (G>A) | rs12412496 (C416286) | 56980 | TaqMan | | | | |
| SNP6 (A>C) | rs2420367 (C11756716) | 19982 | PCR-RFLP | TTTTCCATCCCACTTTCAGC | ACAGGCAGAAAACAGCGAAC | | *SmlI* |
| SNP7 (C>T) | rs4933194 (C375195) | 14816 | TaqMan | | | | |
| SNP8 (A>G) | rs1045949 | 10643 | Direct sequence | AGGGCACTGTCCTTTCAGAC | ATGTCTGCCCTTCAGCCATT | | |

Japan). Further details about primer pairs and culture conditions for the cells are available on request.

## Mutation Search

We performed dHPLC analysis, details of which can be seen in a previous paper (Suzuki *et al*, 2003). Primer pairs were designed using information from the GenBank sequence (accession number: NM-030059.11) and Erdman's paper (Erdmann *et al*, 1996), as well as from 14 amplified regions that covered all the exons, alternative splice exon (exon D), and introns including the branch sites and 1000 bp upstream from initial exon of HTR7.

## Statistical Analysis

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

Marker–trait association analysis was performed with the COCAPHASE 2.403 program (Dudbridge, 2003). This program performs log-likelihood ratio tests under a log-linear model for global *P*-value. To estimate haplotype frequencies, an expectation–maximization (EM) algorithm was used (Zhao *et al*, 2000). Rare haplotypes found in less than 3% of both cases and controls were excluded from association analyses due to the limitation of the EM algorithm. For haplotypic analyses, we calculated global *P*-values in multi-SNP haplotype systems. In both haplotypic and single marker allelewise analyses, we performed the permutation procedure that is a tool in the COCAPHASE program in a 1- to 3-marker sliding window fashion. We emphasized the permutation *P*-values rather than individual global *P*-values, because this permutation method is considered to be appropriate for these analyses (Dudbridge, 2003) (ie Bonferroni correction for multiple testing is considered to be too conservative to apply to genetic association analyses (Nyholt, 2001)). Ten thousand permutations were performed in each permutation procedure, and ORs (ORs) of risk alleles or haplotypes were calculated when the most frequent alleles or haplotypes had ORs of 1. To detect risk haplotypes, we performed the individual procedures in this program. The significance level for all statistical tests was 0.05.

# RESULTS

## LD Mapping

We genotyped eight SNPs for 96 controls. After testing for deviation from HWE (all SNPs were in HWE), we evaluated pairwise LD matrices between each SNP, and selected 'htSNPs' from LD blocks. Consequently, we determined three 'LD blocks' and four 'htSNPs' (SNP1, SNP2, SNP5, and SNP7) (Table 2).

## Association Analysis

We expanded genotyping of these four 'htSNPs' for all the schizophrenia subjects and the remaining 255 controls. In this step, genotype distributions were again in HWE.

**Table 2** Pairwise Linkage Disequilibrium (LD) Matrices and 'LD Blocks' in HTR7

| | | BLOCK1 | | | BLOCK2 | | BLOCK3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Block**[a] | **SNPID** | **SNP1** | **SNP2** | **SNP3** | **SNP4** | **SNP5** | **SNP6** | **SNP7** | **SNP8** | **MAF**[b] **(%)** |
| BLOCK1 | SNP1 | | 1.0 (0.56–1.0) | 1.0 (0.95–1.0) | 0.71 (0.33–0.88) | 0.77 (0.37–0.92) | 0.83 (0.71–0.91) | 0.85 (0.76–0.92) | 0.78 (0.66–0.87) | 32.8 |
| | SNP2 | 0.09 | | 1.0 (0.54–1.0) | 1.0 (0.86–1.0) | 0.90 (0.71–0.97) | 0.77 (0.21–0.93) | 0.82 (0.32–0.95) | 0.76 (0.20–0.93) | 16.1 |
| | SNP3 | 0.98 | 0.09 | | 0.79 (0.42–0.93) | 0.88 (0.48–0.97) | 0.83 (0.72–0.91) | 0.88 (0.78–0.94) | 0.79 (0.66–0.87) | 32.3 |
| BLOCK2 | SNP4 | 0.10 | 0.48 | 0.12 | | 1.0 (0.93–1.0) | 0.78 (0.40–0.92) | 0.82 (0.50–0.94) | 0.79 (0.41–0.92) | 28.6 |
| | SNP5 | 0.11 | 0.42 | 0.14 | 0.93 | | 0.87 (0.47–0.97) | 0.90 (0.57–0.98) | 0.88 (0.48–0.97) | 27.1 |
| BLOCK3 | SNP6 | 0.60 | 0.05 | 0.62 | 0.10 | 0.12 | | 1.0 (0.93–1.0) | 0.95 (0.87–0.99) | 29.7 |
| | SNP7 | 0.73 | 0.06 | 0.75 | 0.13 | 0.15 | 0.86 | | 0.95 (0.86–0.99) | 32.8 |
| | SNP8 | 0.56 | 0.05 | 0.57 | 0.11 | 0.13 | 0.86 | 0.82 | | 30.7 |

[a]Each BLOCK was determined by HAPLOVIEW.
[b]MAF = minor allele frequencies of 96 controls in LD mapping.
Numbers of upper diagonals are D′ and parenthetical numbers are 95% confidential intervals of D′. Numbers of lower diagonals are $r^2$.
Boxes in gray represent 'ht SNPs'.

**Table 3** Association Analyses of Four 'ht SNPs' in HTR7

| | Multi-SNP haplotype systems[a] | | | | Genotypic distribution[b] | | | | | | | Allelic distribution MAF (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | M/M | | M/m | | m/m | | | | |
| **SNPID** | **1SNP** | **2SNP** | **3SNP** | **4SNP** | **SCZ** | **CON** | **SCZ** | **CON** | **SCZ** | **CON** | ***P*-values (genotype)** | **SCZ** | **CON** |
| SNP1 | 0.347 | | | | 177 | 147 | 156 | 157 | 50 | 47 | 0.477 | 33.4 | 35.8 |
| | | **0.0274** | | | | | | | | | | | |
| SNP2 | **0.00571** | | **0.0137** | | 231 | 240 | 124 | 98 | 28 | 13 | **0.0257** | 23.5 | 17.7 |
| | | **0.00584** | | **0.0398** | | | | | | | | | |
| SNP5 | **0.00504** | | **0.0280** | | 162 | 175 | 168 | 149 | 53 | 27 | **0.0128** | 35.8 | 28.9 |
| | | **0.0313** | | | | | | | | | | | |
| SNP7 | 0.664 | | | | 168 | 147 | 159 | 152 | 56 | 52 | 0.856 | 35.4 | 36.5 |
| *Permutation P-value*[c] | ***0.0197*** | ***0.0386*** | ***0.0424*** | | | | | | | | | | |

[a]*P*-values were calculated by log-likelihood ratio test (1SNP; allelewise association, SNP2–4; global haplotypic association). Bold numbers represent significant *P*-values. Bold italics numbers represent significant *P*-values of permutation test.
[b]M = major allele; m = minor allele; SCZ = schizophrenia; CON = control; MAF = minor allele frequency.
[c]An implement in the program COCAPHASE.

Almost all global *P*-values except 1-marker global *P*-values of SNP1 and SNP7 were associated with schizophrenia. After adjustment by permutation procedure, we found a significant association of all permutation *P*-values with schizophrenia (Table 3). An assessment of the components of these significant associations indicated SNP2 and SNP5 as possible key components for schizophrenia ('A' allele of SNP2, case = 23.5%, control = 17.7%, and *P*-value = 0.00571; 'A' allele of SNP5, case = 35.8%, control = 28.9%, and *P*-value = 0.00504; Table 3). The ORs for the significant risk genotypes are modest; OR = 1.43 (95% CI: 1.11–1.85) for SNP2, OR = 1.37 (95% CI: 1.10–1.71) for SNP5.

To detect the risk haplotypes, individual haplotypic analyses were performed from positive global *P*-values (Table 4). The most significant haplotype overtransmitted in schizophrenia was a combination of SNP2 and SNP5 ('A–A' haplotype, case = 23%, control = 16%, and *P*-value = 0.0015); however, the estimated haplotype frequencies of each risk haplotype were nearly equal to the minor allele frequency (MAF) of SNP2 (case = 23.5%, control = 17.7%).

## Dual-Luciferase Reporter Assay of SNP2

Taken together with the results of the LD mapping (SNP2 was in strong LD with SNP5), we hypothesized that SNP2, a possible promoter SNP (533 bp from initial exon), may be a susceptibility SNP for schizophrenia. To confirm the functional relevance of SNP2, we developed a dual-luciferase reporter assay. However, the results from this assay did not provide evidence that SNP2 modifies transcriptional activity of HTR7 (*P*-value = 0.782; RLAwt = 100 ± 58.2, RLAmt = 86.2 ± 45.2).

**Table 4** Individual Haplotypic Analyses from Positive Permutation Analyses of Schizophrenia

| Combination of SNPs | Marker haplotype | Frequency | | | |
|---|---|---|---|---|---|
| | | **SCZ** | **CON** | ***P*-values** | **OR** |
| SNP1-2 | C–G | 0.43 | 0.47 | NS | 1 |
| | C–A | 0.23 | 0.18 | 0.0068 | 1.42 |
| | T–G | 0.33 | 0.36 | NS | |
| SNP2-5 | G–G | 0.64 | 0.70 | 0.013 | 1 |
| | G–A | 0.13 | 0.13 | NS | |
| | A–A | 0.23 | 0.16 | 0.0015 | 1.54 |
| SNP5-7 | G–C | 0.32 | 0.37 | 0.043 | 1 |
| | G–T | 0.32 | 0.34 | NS | |
| | A–C | 0.33 | 0.27 | 0.010 | 1.41 |
| SNP1-2-5[a] | C–G–G | 0.33 | 0.37 | NS | 1 |
| | C–A–A | 0.23 | 0.16 | 0.0015 | 1.59 |
| SNP2-5-7[a] | G–G–C | 0.32 | 0.35 | NS | 1 |
| | A–A–T | 0.21 | 0.15 | 0.0035 | 1.59 |
| SNP1-2-5-7[a] | C–G–G–C | 0.30 | 0.34 | NS | 1 |
| | C–A–A–C | 0.21 | 0.15 | 0.0041 | 1.57 |

SCZ = schizophrenia; CON = control; NS = not significant; OR = odds ratio.
[a]Only reference haplotypes and positive haplotypes are shown.

## Mutation Search

To detect the actual susceptibility variants, we performed a systematic mutation search in all exons and introns including branch sites and 1000 bp upstream from the initial exon. However, we could not find any functional mutations in these regions.

## DISCUSSION

In this study, two htSNPs (a possible promoter SNP (SNP2) and an intronic SNP (SNP5)) and haplotypes showed an association with schizophrenia in a Japanese population. However, the functional relevance of SNP2 could not be confirmed with the dual-luciferase reporter assay.

The method of using 'htSNPs' in LD association analyses is more sensitive and powerful than that of using randomly selected SNPs. Here, selection of 'htSNPs' resulted in the fitting of another criteria, 'LD-selected htSNPs', at a relatively stringent $r^2$ threshold ($r^2 > 0.8$) (Carlson *et al*, 2004). This method of 'LD-selected SNPs' reflects the evolutionary relationships of haplotypes. Therefore, our 'htSNPs' could identify disease associations with either specific haplotypes or with clades of related haplotypes (Carlson *et al*, 2004). Given this LD pattern of HTR7, the actual susceptibility variants may exist anywhere in HTR7.

Although our sample size in this mutation search was sufficient to detect common variants with more than 5% MAF at 95% power (Collins and Schwartz, 2002), we failed to find the actual susceptibility variants through a systematic mutation search. Since the actual promoter region in HTR7 has not been determined, the regions targeted in our mutation search might not have been adequate. In addition, we did not search the conserved noncoding sequence that plays a role in gene regulation. Further investigation will be required for conclusive results.

An additional point deserves attention in interpreting the results: The positive association with schizophrenia could be due to type I error, possibly because of population stratification or unmatched age samples. Replication study with genomic control or a family-based population will be required.

In conclusion, we found the first, and a significant, association of SNPs in HTR7 with Japanese schizophrenia patients after consideration of problems in statistical genetics. These results support the supposition that HTR7 is a schizophrenia-susceptibility gene.

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# No association was found between a functional SNP in ZDHHC8 and schizophrenia in a Japanese case–control population

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**Abstract**

ZDHHC8 is a new and attractive candidate for a schizophrenia-susceptibility factor. First, several lines of linkage studies showed that 22q11, on which ZDHHC8 is located, is a "hot" region. Second, fine linkage disequilibrium mapping revealed a significant association around ZDHHC8. Moreover, a very recent study reported that one single nucleotide polymorphism (SNP: rs175174) in ZDHHC8 might affect the splicing process, the ZDHHC8 knock-out mice showed the gender-specific phenotype, and the transmission disequilibrium test (TDT) using this SNP also showed significant association with human female schizophrenia. Thus, we attempted a replication study of this SNP using relatively large Japanese case–control samples (561 schizophrenics and 529 controls). No association was found between schizophrenia and controls even after dividing samples by gender. Because our sample size provided quite high power, ZDHHC8 may not play a major role in Japanese schizophrenia. And our results did not support the gender-specific effect of this SNP.
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*Keywords:* Chromosome 22q11; Gender difference; Candidate gene

The 22q11 region (OMIM: #600850 SCZD4) is associated with increased risk for schizophrenia [2]. Two independent meta-analyses of linkage studies showed the linkage around 22q11 [1,5], although one negative result was also reported [8]. This chromosome region contains at least three genes, COMT [12], PRODH2 and DGCR6 [7], implicated as susceptibility genes for schizophrenia.

Recently, ZDHHC8 was reported as a new and attractive candidate gene on 22q11 from the evidence of a genetic association study and animal study [6,9]. In the initial genetic association study, Liu et al. showed that three single nucleotide polymorphisms (SNPs) in ZDHHC8 were associated with schizophrenia. One of these SNPs (rs175174), which was located in intron 4 of ZDHHC8, showed the most highly significant *P* value [6]. This intronic SNP seemed to modify ZDHHC8 expression by causing imperfect splicing, intron retention and reduced enzyme activity. In addition, *Zdhhc8* knockout mice had a gender-dependent dimorphic deficit in prepulse inhibition similar to schizophrenia and reactivity to the psychomimetic *N*-methyl-D-aspartate (NMDA) receptor blocker dizocilpine. In the light of these findings, the transmission disequilibrium test (TDT) divided samples according to gender differences, revealing that human female schizophrenia was significantly associated with this SNP [9]. Thus, we here provide a replication study of rs175174 in ZDHHC8 using Japanese case–control samples.

A total of 561 patients with schizophrenia (259 female; mean age ± standard deviation (S.D.) 49.6 ± 16.4 years: 302

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male; 47.0 ± 14.9) and 529 controls (270 female; 39.7 ± 15.4 years: 259 male; 34.9 ± 12.4 years) were genotyped for association analysis of rs175174. Moreover, in additional linkage disequilibrium (LD) mapping around this SNP, 95 schizophrenic patients (50 female and 45 male) and 96 controls (44 female and 52 male), part of each sample used in association analysis, were genotyped for three SNPs. The general characterization of these subjects and a description of their psychiatric assessment according to identical criteria were published elsewhere [13]. After explaining the study to all subjects, written informed consent was obtained from each. This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine and Fujita Health University.

Genomic DNA was extracted from peripheral blood of all subjects. For rapid genotyping of SNPs, rs175174 and additional three SNPs for LD mapping (rs175169, rs175175 and rs2292570), polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assays were developed. The information of PCR primers is available on request. The PCR reactions of all SNPs were carried out in a 10 μl volume containing 10 ng genomic DNA, 0.4 M of each primer, 200 μM of dNTP, 1× PCR Gold Buffer, 1.5 mM $MgCl_2$ and 0.25 U of Amplitaq Gold™ (Applied Biosystems Japan Ltd., Tokyo, Japan), using the GeneAmp™ PCR system 9700 (Applied Biosystems Japan Ltd.). PCR cycling conditions consisted of an initial denaturation step at 95 °C for 9 min, followed by 45 cycles of 95 °C for 15 s, 56 °C for 20 s, 72 °C for 30 s, and ending with a final extension step at 72 °C for 7 min. PCR product was digested using appropriate restriction enzymes according to the manufacture's recommendation (New England Biolabs, England, UK) (Table 1). DNA fragments were resolved by electrophoresis in a 6% acrylamide gel stained with ethidium bromide.

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by $\chi^2$ test. Marker-trait association analysis was also evaluated by $\chi^2$ test (SPSS 10.0J, SPSS Japan Inc., Japan). To evaluate pairwise LD matrices among SNPs (by $D'$ and $r^2$), we used the software HAPLOVIEW version 2.05 (developed in Mark Daly's lab., URL; http://www.broad.mit.edu/personal/jcbarret/haploview/index.php). This software also defined "LD blocks" by reasonable criteria based on 95% confidential bounds on $D'$ values [4]. Power calculation was performed using a statistical program prepared by Ohashi et al. [10]. The significance level for all statistical tests was 0.05.

In view of the gender differences in gene effects, we included analyses of samples divided according to the gender. Both in cases and controls, genotype frequencies of total, female and male samples were not significantly different from HWE.

In association analysis, we could not find associations of rs175174 with schizophrenia in either male or female (Table 2).

Next, to test whether rs175174 is representative for ZDHHC8 or not, we performed LD mapping using three additional SNPs around ZDHHC8 (Fig. 1). LD matrices between each pair of SNPs showed strong LD both in cases and controls (Table 1). Even after dividing samples according to the gender, all LD patterns showed the same trends (data not shown). These findings may suggest that the LD pattern of ZDHHC8 is a block-like pattern and that rs175174 is the "representative SNP" of this gene.

The power based on genotype relative risk (GRR) was calculated to evaluate the non-significant results due to type II error. When we set the GRR at 1.28, 1.42 and 1.40 in all, female and male samples, respectively (multiplicative model), our sample size provided powers of more than 80%.

We could not replicate an original positive association using TDT of ZDHHC8 with schizophrenia by the present case–control association analysis among Japanese. Nor could we replicate the gender-specific effect of the risk SNP. In this association analysis, our sample sizes provide enough power to deny the hypothesis. We also performed the fine LD mapping of Japanese samples and showed that the LD pattern of ZDHHC8 was the same block-like pattern as one of the samples from the United States and South Africa. The results provide evidence that not only rs175174 but also ZDHHC8 would not be a susceptibility factor for schizophrenia in either Japanese females or males. The discrepancy between Japanese and the samples from the United States and South Africa may derive from ethnic differences.

A couple of limitation should be addressed to discuss the present results. Initially, the mean age of controls is much younger than that of patients in the present study. This means that a number of young controls, although not more than five subjects given a lifetime morbidity risk of 0.8–1.0%, may go on to develop schizophrenia. This confounding factor might weaken the power of the present study. Another limitation

Table 1
SNPs in LD mapping and pairwise LD matrices

| SNP ID | $D'$ | | | | Restriction enzyme |
|---|---|---|---|---|---|
| | rs175169 | rs175174 | rs175175 | rs2292570 | |
| rs175169 | | 0.97 (0.78) | 1.0 (0.29) | 1.0 (0.67) | *Bsl*I |
| rs175174 | 0.97 (0.80) | | 1.0 (0.36) | 1.0 (0.58) | *Bse*RI |
| rs175175 | 1.0 (0.26) | 1.0 (0.31) | | 1.0 (0.21) | *Alw*NI |
| rs2292570 | 0.93 (0.76) | 0.97 (0.70) | 1.0 (0.23) | | *Tsp*RI |

Upper diagonal figures are $D'$ ($r^2$) of controls and lower diagonal figures are $D'$ ($r^2$) of schizophrenia.

Table 2
Association analysis of rs175174

| Samples | Number | G/G | G/A | A/A | P value (genotype) | MAF[a] | P value (allele) |
|---|---|---|---|---|---|---|---|
| Total | | | | | | | |
| SCZ | 561 | 238 | 245 | 78 | | 0.357 | |
| CON | 529 | 205 | 259 | 65 | 0.213 | 0.368 | 0.618 |
| Female | | | | | | | |
| SCZ | 259 | 114 | 106 | 39 | | 0.355 | |
| CON | 270 | 112 | 130 | 28 | 0.133 | 0.344 | 0.714 |
| Male | | | | | | | |
| SCZ | 302 | 124 | 139 | 39 | | 0.359 | |
| CON | 259 | 93 | 129 | 37 | 0.457 | 0.392 | 0.260 |

SCZ: schizophrenia; CON: control.
[a] Minor allele frequency.

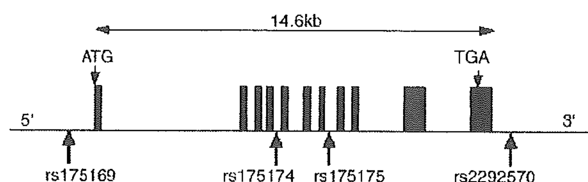


Fig. 1. Genomic structure of ZDHHC8 and SNPs used in association analysis and LD mapping. Vertical bars represent exons of ZDHHC8, and each number under arrows represents SNP ID.

which must be exercised is that the other candidates related to the neurodevelopmental and neuroprotective effect of ZDHHC8 would be in locus heterogeneity [11]. For example, ZDHHC8 encodes a putative transmembrane palmitoyltransferase modulating numerous classes of neuronal proteins including proteins important for neuronal development, neurotransmitter receptors such as NMDA [3]. Thus, the combined effect between ZDHHC8 and the other genes might be a stronger predisposing factor. Further genetic analysis including related candidate genes would definitely be required for a conclusive result.

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# A nonsynonymous polymorphism in the human fatty acid amide hydrolase gene did not associate with either methamphetamine dependence or schizophrenia

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**Abstract**

Genetic contributions to the etiology of substance abuse and dependence are topics of major interest. Acute and chronic cannabis use can produce drug-induced psychosis resembling schizophrenia and worsen positive symptoms of schizophrenia. The endocannabinoid system is one of the most important neural signaling pathways implicated in substance abuse and dependence. The fatty acid amide hydrolase (FAAH) is a primary catabolic enzyme of endocannabinoids. To clarify a possible involvement of FAAH in the etiology of methamphetamine dependence/psychosis or schizophrenia, we examined the genetic association of a nonsynonymous polymorphism of the FAAH gene (Pro129Thr) by a case-control study. We found no significant association in allele and genotype frequencies of the polymorphism with either disorder. Because the Pro129Thr polymorphism reduces enzyme instability, it is unlikely that dysfunction of FAAH and enhanced endocannabinoid system induce susceptibility to either methamphetamine dependence/psychosis or schizophrenia.
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*Keywords:* The fatty acid amide hydrolase (FAAH); Methamphetamine dependence/psychosis; Schizophrenia; Nonsynonymous polymorphism; The Pro 129Thr polymorphism

In 1988, the existence of a cannabinoid receptor in the brain was found, and its gene was cloned two years later [7,19]. To date, at least two different cannabinoid receptors, CB1, CB2, and putative endogenous agonists, including anandamide and 2-arachidonylglycerol, have been identified [8]. CB1 receptors are the only cannabinoid receptors that have been found in the central nervous system (CNS). A number of studies

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have indicated that brain CB1 receptors mediate the behavioral and neurochemical properties of cannabis, e.g., marijuana, including the rewarding effect, tolerance, and physical dependence [9,24,30]. CB1 receptors and endogenous agonists are involved not only in cannabinoid dependence but also dependence on other classes of drugs, such as alcohol, morphine, and cocaine [11,22,23,38]. Interaction between cannabinoids and amphetamines, including methamphetamine, has also been reported. Thus, the cannabinoid receptor antagonist AM251 inhibited methamphetamine self-administration by reduction of methamphetamine withdrawal [37]. Another antagonist, SR141716A, potentiated the stimulating effects of amphetamine [18]. The CB1 agonist WIN 55, 212-2 or delta 9-tetrahydrocannabinol, a main ingredient of cannabis, induced cross-sensitization to amphetamine [14,21]. These findings implicate the endocannabinoid system as one of the most important signaling pathways in drug abuse and dependence.

In the 1980s, fatty acid amide hydrolase (FAAH), the integral membrane cannabinoid enzyme, was identified [27]. FAAH is widely expressed in neuronal cells in the CNS, predominantly in the neocortex, hippocampal formation, amygdala, and cerebellum [10,31]. FAAH serves as a primary catabolic regulator of the endogenous cannabinoid ligand anandamide and related fatty acid amide-signaling molecules [15,16]. It has been reported that the metabolic activity of FAAH plays important roles in the CNS by ensuring rapid termination of specific signaling processes of the cannabinoid system [3–5]. Mice lacking FAAH (FAAH −/− mice) are severely impaired in their ability to degrade anandamide. As a consequence, the brain level of anandamide in FAAH −/− mice is increased 15-fold, and they exhibit CB1 receptor-dependent behavioral responses, including less sensitivity to several pain stimuli, hypomotility, hypothermia, analgesia, and catalepsy [2]. Mutation of the FAAH gene may induce dysregulation of the endogenous cannabinoid system, and result in alternation in brain addiction/reward pathways. Recently, Sipe et al. [28] reported that the presence of a polymorphism of the FAAH gene that converts a conserved proline residue to threonine at the 129 position (Pro129Thr) is a risk factor for problem substance abuse and dependence in a Caucasian population. We tried to determine if a genetic association of the Pro129Thr nonsynonymous polymorphism of the FAAH gene in patients with methamphetamine dependence in a Japanese population.

Furthermore, acute and chronic cannabis exposure can precipitate a psychotic state, with hallucinations and delusions resembling schizophrenia. Cannabis consumption also worsens positive symptoms of schizophrenia, and could result in a poor outcome and liability to relapse [12,13,20,29,32]. Two cohort studies showed cannabis use in adolescence increased greatly the risk of schizophrenia in adulthood [1,39]. In addition, recent studies showed an increased density of CB1 receptors in the prefrontal cortex and an increased level of anandamide in the CSF in schizophrenia [6,17,40]. These findings led to a cannabinoid hypothesis of schizophrenia [34]. Therefore, we examined a possible association between Pro129Thr polymorphism of the FAAH gene and schizophrenia.

The subjects were 153 patients with methamphetamine dependence (124 males and 29 females; mean age, 37.8 years; S.D. 12.1 years) meeting the ICD-10-DCR criteria (F15.2), who were outpatients or inpatients in psychiatric hospitals of the Japanese Genetics Initiative for Drug Abuse (JGIDA), and 200 age-, gender-, and geographical origin-matched normal controls (designated Control-1, 162 males and 38 females; mean age, 37.3 years; S.D. 12.1 years), who were mostly medical staff members without a past individual or family history of drug dependence or psychotic disorders. One hundred and forty-one of patients also suffered from methamphetamine psychosis (F15.5, 118 males and 23 females; mean age, 38.5 years; S.D. 12.1 years). The patients with methamphetamine dependence and/or psychosis were divided into several subgroups by clinical features according to age at first consumption, latency of psychosis, prognosis, and multi-substance abuse status. Seventy-six patients (49.7%) had consumed methamphetamine before the age 20 years, and 77 patients (50.3%) had first consumed methamphetamine after they were 20 years old. The latency of psychosis was less than 3 years after the first methamphetamine consumption in 60 patients (42.6%) and 3 or more years in 81 patients (57.4%). Forty-eight patients (31.4%) had abused only methamphetamine during their lifetime, and 105 patients (68.6%) had abused drugs other than methamphetamine in the past or present. Besides methamphetamine, organic solvents were the most frequently abused drugs, followed by marijuana. Cocaine and heroin were rarely abused. The prognosis of methamphetamine psychosis varied among patients, and some patients showed continuous psychotic symptoms even after methamphetamine discontinuance, as previously reported [25,26]. Therefore, patients were divided into two categories of psychosis, the transient type and the prolonged type, based on the duration of the psychotic state after methamphetamine discontinuance, as described in our previous study [36]. Patients with the transient type showed remission of psychotic symptoms within one month after the discontinuance of methamphetamine consumption and beginning of treatment with neuroleptics, and those with the prolonged type had psychosis that continued for more than one month even after this discontinuance of methamphetamine consumption and beginning of neuroleptic treatment. In this study, 85 patients (60.3%) were the transient type and 56 patients (39.7%) were the prolonged type. It has been well-documented that once methamphetamine psychosis has developed, patients in the remission state become liable to spontaneous relapse without reconsumption of methamphetamine [25,26,35]. Such enhanced liability to relapse may result from a sensitization phenomenon developed during methamphetamine abuse, and may be affected by genetic traits [33]. Therefore, the patients were divided into two groups according to the presence or absence of spontaneous relapse. The number of patients with a history of

spontaneous relapse was 65 (46.1%) and those without were 76 (53.9%).

To determine the relationship between the Pro129Th polymorphism and schizophrenia, we examined 260 patients (151 males and 109 females; mean age, 44.5 years; S.D. 13.1 years) fulfilling the ICD-10 diagnostic criteria for schizophrenia. Assessment for diagnosis and subtype of schizophrenia was performed by trained psychiatrists on the basis of all available information, including hospital notes. One hundred and twenty-seven patients (68 males and 59 females; mean age, 39.1 years; S.D. 13.1 years) were diagnosed with the paranoid type, 127 (82 males and 45 females; mean age, 39.6 years; S.D. 13.9 years) with the hebephrenic type, 2 with the catatonic type, and 4 residual type of schizophrenia. Age-, gender-, and geographical origin-matched control subjects for the schizophrenia patients were recruited (designated Control-2, 194 males and 143 females; mean age, 47.2 years; S.D. 11.8 years). Subjects with a positive personal or familial history of major psychiatric disorders were excluded from the control group. This study was performed after obtaining approval from the ethics committees of each institute of JGIDA, and all subjects provided written informed consent for the use of their DNA samples in this research.

The genomic DNA was extracted from peripheral leukocytes using the standard phenol/$CHCl_3$ method. A Pro129Thr polymorphism of the FAAH gene was amplified by polymerase chain reaction (PCR), with 3% dimethyl sulfoxide and 0.75 units of Taq DNA polymerase in a total volume of 15 μl reaction mixture using the following primer sets: 5′-ATG TTG CTG GTT ACC CCT CTC C-3′ and 5′-TCA CAG GGA CGC CAT AGA GCT G-3′. Initial denaturation was performed for 5 min at 95 °C. Then, 35 cycles were performed (30 s of denaturing at 95 °C, 30 s of annealing at the appropriate temperature, and 30 s of extension at 72 °C), followed by a final extension at 72 °C for 5 min. The PCR products were then digested with EcoO109I and analyzed on 3.0% agarose gels.

Deviation of the genotype counts from Hardy–Weinberg equilibrium was tested using a chi-square goodness-of-fit test. The statistical significance of differences in the genotype distribution and allele frequency between patients and controls was assessed by a chi-square test or Fisher's exact test at a significance level of 0.05. All genotyping was performed in a blinded fashion, with the control and case samples mixed randomly. Allele frequencies were calculated using allele-counting methods.

The genotype distribution and allele frequencies of Pro129Thr polymorphism of the FAAH gene for patients with methamphetamine dependence/psychosis or schizophrenia, Control-1, and Control-2 are shown in Tables 1 and 2. The genotype distribution of all patients and controls subjects did not deviate significantly from Hardy–Weinberg equilibrium at the polymorphic locus. No significant differences were found in the frequency of the genotype or allele of the Pro129Thr polymorphism between patients with methamphetamine dependence and Control-1 (genotype, $\chi^2 = 1.22$, d.f. = 2, $P = 0.57$; allele, $\chi^2 = 0.22$, d.f. = 1, $P = 0.68$). No significant differences were found in the frequency of the genotype or allele between subcategories of methamphetamine-dependent patients whose age at first methamphetamine consumption was less 20 years or more than 20 years (genotype, $\chi^2 = 1.80$, d.f. = 2, $P = 0.45$; allele, $\chi^2 = 0.65$, d.f = 1, $P = 0.45$). Nor was there a significant difference between patients with and without multiple substance abuse (genotype, $\chi^2 = 2.01$, d.f. = 2, $P = 0.49$; allele, $\chi^2 = 0.60$, d.f = 1, $P = 0.52$), or patients whose latency of methamphetamine-induced psychosis was less and more than 3 years (genotype, $\chi^2 = 0.66$, d.f. = 2, $P = 0.75$; allele, $\chi^2 = 0.51$, d.f = 1, $P = 0.52$), or between patients with transient and prolonged psychosis (genotype, $\chi^2 = 3.26$, d.f. = 2, $P = 0.19$; allele, $\chi^2 = 3.08$, d.f = 1, $P = 0.11$), or patients with and without spontaneous relapse of psychotic symptoms (genotype, $\chi^2 = 4.56$, d.f. = 2, $P = 0.16$; allele, $\chi^2 = 4.36$, d.f = 1, $P = 0.06$).

No significant differences were found in the frequency of the genotype or allele of the Pro129Thr polymorphism between schizophrenia patients and Control-2 (genotype, $\chi^2 = 0.18$, d.f. = 2, $P = 0.91$; allele, $\chi^2 = 0.01$, d.f. = 1, $P = 0.94$). With regard to the subcategories of schizophrenia, no significant differences were found in the frequency of the genotype or allele between patients with paranoid type schizophrenia and Control-2 (genotype, $\chi^2 = 0.67$, d.f. = 2, $P = 0.63$; allele, $\chi^2 = 0.02$, d.f = 1, $P = 0.92$), or patients with hebephrenic type schizophrenia and Control-2 (genotype, $\chi^2 = 1.15$, d.f. = 2, $P = 0.61$; allele, $\chi^2 = 0.32$, d.f. = 1, $P = 0.62$).

FAAH is a primary and rapid catabolizer of endocannabinoids, such as anandamide and 2-arachidonoyl glycerol, and FAAH knockout mice show a robust increase in brain anandamide. Therefore, homozygosity of a mutant allele of the gene may induce hyperactivity of cannabinoid signaling in the brain. The present study showed no significant association between methamphetamine dependence/psychosis and the Pro129Thr nonsynonymous polymorphism of the FAAH gene. Sipe et al. [28] reported that the Pro129Thr polymorphism is strongly associated with street-drug use and problem drug/alcohol use, especially with illegal drug use by Caucasians. They found that the odds ratios of the mutant 129Thr/Thr homozygote for risk of problem drug/alcohol use and street-drug use in individuals were 4.5 and 2.2, respectively [28]. The Pro129Thr mutation did not significantly impact the catalytic properties of FAAH, but it was found to produce a significantly greater sensitivity to proteolytic degradation, and may have direct effects on the regulation of the FAAH proteins [28]. These findings suggest that dysfunction of FAAH and enhanced endocannabinoid level due to genetic mutation may constitute a risk factor for problem drug use. However, our data indicated that dysfunction of FAAH did not affect the risk of methamphetamine dependence/psychosis in a Japanese population. We also examined a possible association between clinical features of methamphetamine dependence/psychosis, such as age at first methamphetamine

Table 1
Fatty acid amide hydrolase (FAAH) genotype distributions and allele frequency in patients with methamphetamine (METH) dependence/psychosis

| Group | N | Genotype | | | | Allele | | |
|---|---|---|---|---|---|---|---|---|
| | | Pro/Pro (%) | Pro/Thr (%) | Thr/Thr (%) | P-value | Pro (%) | Thr (%) | P-value |
| Control-1 | 200 | 139 (69.5) | 58 (29.0) | 3 (1.5) | | 336 (84.0) | 64 (16.0) | |
| METH dependence/psychosis | 153 | 105 (68.6) | 43 (28.1) | 5 (3.3) | 0.57 | 253 (82.7) | 53 (17.3) | 0.68 |
| Age of first use | | | | | | | | |
| <20 years | 76 | 49 (64.5) | 25 (32.9) | 2 (2.6) | | 123 (81.0) | 29 (19.1) | |
| ≥20 years | 77 | 56 (72.7) | 18 (23.4) | 3 (3.9) | 0.45 | 130 (84.4) | 24 (15.6) | 0.45 |
| Multi-substance abuse | | | | | | | | |
| No | 48 | 30 (62.5) | 17 (35.5) | 1 (2.1) | | 77 (80.2) | 19 (19.8) | |
| Yes | 105 | 75 (71.4) | 26 (24.8) | 4 (3.8) | 0.49 | 176 (83.8) | 34 (16.2) | 0.52 |
| Latency of psychosis | | | | | | | | |
| <3 years | 60 | 43 (71.7) | 16 (26.7) | 1 (1.7) | | 102 (85.0) | 18 (15.0) | |
| ≥3 years | 81 | 54 (66.7) | 24 (29.6) | 3 (3.7) | 0.75 | 132 (81.5) | 30 (18.5) | 0.52 |
| Prognosis of psychosis | | | | | | | | |
| Transient | 85 | 53 (62.4) | 29 (34.1) | 3 (3.5) | | 135 (79.4) | 35 (20.6) | |
| Prolonged | 56 | 43 (76.8) | 12 (21.4) | 1 (1.8) | 0.19 | 98 (87.5) | 14 (12.5) | 0.11 |
| Spontaneous relapse of pyschotic symptoms | | | | | | | | |
| No | 76 | 47 (61.8) | 26 (34.2) | 3 (3.9) | | 120 (78.9) | 32 (21.1) | |
| Yes | 65 | 50 (76.9) | 14 (21.5) | 1 (1.5) | 0.16 | 114 (87.7) | 16 (12.3) | 0.06 |

Numbers in parentheses indicate percentages. Statistical analysis was performed by a chi-square test of Fisher's exact test.

Table 2
FAAH genotype distributions and allele frequency in schizophrenia patients

| Group | N | Genotype | | | | Allele | | |
|---|---|---|---|---|---|---|---|---|
| | | Pro/Pro (%) | Pro/Thr (%) | Thr/Thr (%) | P-value | Pro (%) | Thr (%) | P-value |
| Control-2 | 337 | 233 (69.1) | 99 (29.4) | 5 (1.5) | | 565 (84.0) | 109 (16.0) | |
| Schizophrenia | 260 | 180 (69.2) | 75 (28.8) | 5 (1.9) | 0.91 | 435 (83.7) | 85 (16.3) | 0.94 |
| Paranoid type | 127 | 90 (70.9) | 34 (26.8) | 3 (2.4) | 0.63 | 214 (84.3) | 40 (15.7) | 0.92 |
| Hebephrenic type | 127 | 83 (65.4) | 43 (33.9) | 1 (0.0) | 0.61 | 209 (82.3) | 45 (17.7) | 0.62 |

Numbers in parentheses indicate percentages. Statistical analysis was performed by a chi-square test of Fisher's exact test.

consumption, latency to onset of psychosis, prognosis, spontaneous relapse, and multi-substance abuse status but found no significant association with any clinical feature. The discrepancy between the previous and present findings may result from differences of substance class. The present study analyzed methamphetamine abusers, however, Spine et al. analyzed "street-drug users". Although, they did not specify the kinds of drugs in their paper, marijuana use has been epidemic in the US, followed by cocaine and morphine. It is possible that the majority of patients examined in the previous study abuse marijuana and that is why the "street-drug use" of the previous study was associated with a mutant allele of the FAAH gene. This hypothesis should be addressed in future study. Alternatively, the ethnicity of the subjects must be considered. Our subjects were Japanese, and theirs were Caucasian. Japanese controls showed the 129Thr allele at 14.6–16.0%, compared to Caucasians at 28.3–30.6%. Homozygosity of the mutant allele was observed in only 1.5% of Japanese subjects. The rarity of the mutant homozygote in our Japanese population may result in a lack of genetic risk of the FAAH gene for Japanese "street-drug users".

No significant association with the Pro129Thr nonsynonymous polymorphism of the FAAH gene with schizophrenia was revealed. This is consistent with Sipe's study of a Caucasian population. Previously, we reported that a triplet repeat polymorphism of the CNR1 gene, which encodes the human CB1 cannabinoid receptor, was significantly associated with patients with schizophrenia, especially the hebephrenic subtype [36]. Several clinical studies have shown that exogenous cannabinoid ligands could precipitate schizophrenia, worsen prognosis, and induce relapse. Our present and previous genetic findings of the endocannabinoid system indicated that variants of cannabinoid receptors, rather than an altered endogenous agonist produced by the FAAH variant, may be important in the etiology of schizophrenia.

The power analysis showed that the present sample size had a power of 0.89 and 0.98 to detect a small effect size ($w$ = 0.12) at an alpha value of 0.05 to detect significant allelic associations between Control-1 and total methamphetamine patients and between Conrol-2 and total schizophrenic patients, respectively. The present total sample size can be therefore considered to be large enough statistically. However, the

statistical power deteriorated in the analysis with regard to the subgroups of patients, and our results must be verified with a larger sample to conclude.

In conclusion, the cannabinoid pathway may be implicated in drug abuse, addiction, and also the pathophysiology of schizophrenia, but the Pro129Thr nonsynonymous polymorphism of the FAAH gene is not significantly associated with either methamphetamine dependence/psychosis or schizophrenia, at least in a Japanese population.

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# No association between monoamine oxidase A promoter polymorphism and personality traits in Japanese females

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**Abstract**

Monoamine oxidase A (MAO-A) is an enzyme involved in the metabolism of monoamine neurotransmitters such as dopamine, serotonin, and noradrenaline in the brain. Previous studies have demonstrated a significant association between MAO-A gene polymorphism and personality traits in males. The purpose of the present study was to examine this association in females. The subjects were 219 healthy Japanese females. We genotyped a variable number of tandem repeats located upstream of the MAO-A gene. Personality traits were assessed using the Temperament and Character Inventory (TCI). There was no association between any personality trait and MAO-A genotype. The present results do not support the hypothesis that MAO-A gene polymorphism is related to certain personality traits in females.
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*Keywords:* Monoamine oxidase A; Personality; TCI; Polymorphism; Gene

Monoamine oxidase (MAO) is a mitochondrial enzyme that catalyzes the degradation of several different biological amines, and includes two distinguishable forms: MAO-A and MAO-B. The former is present in the catecholaminergic neurons of the brain, and has a preference for noradrenaline, adrenaline, and serotonin as substrates, whereas the latter is present in the serotonergic neurons of the brain, and prefers β-phenylethylamine as a substrate. Both forms can oxidize dopamine, tyramine, and tryptamine [13].

According to Cloninger's model, human personality consists of seven dimensions including three temperament dimensions and four character dimensions, and on the basis of this model Cloninger developed the Temperament and Character Inventory (TCI), a questionnaire for assessing personality traits [2]. The three temperament dimensions, which include Novelty Seeking, Harm Avoidance, Reward Dependence, and Persistence, have been assumed to be related to monoamine neurotransmitters: Novelty Seeking with dopaminergic activities, Harm Avoidance with serotonergic activities, and Reward Dependence with noradrenergic activities (Table 1). Since MAO is involved in the metabolism of these monoamine neurotransmitters, this enzyme in the brain might be closely related to human personality measured by the TCI.

Additionally, Brunner et al. [1] reported the Brunner syndrome, caused by a deficiency in MAO-A, which has features of borderline mental retardation and repetitive violent behavior. The restriction of dopaminergic activities resulting from MAO-A deficiency is considered to contribute to the aggressive behavior. Following this, there was an increased number of studies that investigated the relationship between the MAO-A gene and behavioral traits.

MAO-A is encoded by a single gene located on bands Xp11.23. A polymorphism located 1.2 kb upstream of the MAO-A coding sequences has been shown to affect the

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