Very Long Duration of Vulnerability

The last characteristic of the sensitization observed in patients with METH psychosis is the very duration of vulnerability to the relapse of psychosis, which is the most serious clinical issue in the therapy for substance abuse. A patient who is in remission from METH psychosis even for several years or decades can easily relapse due to a few injections of METH. Sato *et al.*[10] reported a patient who developed a rapid relapse of psychosis after only a single METH injection after abstinence for 60 months. Therefore, the vulnerability to the relapse of psychosis developed during METH abuse does not seem to decrease over time.

CONCLUSION

As shown in FIGURE 3,[6] two other distinct conditions, chronic schizophrenia in humans and behavioral sensitization in rodents, showed temporal profiles quite similar to that of METH dependence and psychosis. This implies that these conditions share a common molecular mechanism, the sensitization phenomenon. Basic studies using animal models of behavioral sensitization have provided many divergent neurochemical and molecular findings[6,11] that probably underlie the mechanisms of sensitization. Findings derived from animal models are very useful for understanding the onset process, relapse mechanisms, and chronic and refractory courses in schizophrenia and METH dependence and psychosis, and may open the way for development of innovative and radical therapies for them.

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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.

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 χ^2 test. Marker-trait association analysis was also evaluated by χ^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)	*Ahw*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.

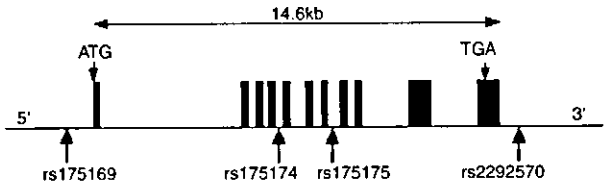

Fig. 1. Genomic structure of ZDHHC8 and SNPs used in association analysis and LD mapping. Vertical bars represent exons of ZDHHC8, and each number under arrows represents SNP ID.

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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Short Communication

Novel missense polymorphism in the regulator of G-protein signaling 10 gene: analysis of association with schizophrenia

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ABSTRACT Dysfunction of neuronal signal transduction via G-protein has previously been speculated to be involved in the pathophysiology of schizophrenia. Regulator of G-protein signaling (RGS) is a protein that acts as a GTPase-activator for Gα protein. A total of 33 Japanese patients with schizophrenia were screened for mutations in the coding region of the RGS10 gene, and a novel missense polymorphism (Val38Met) in the RGS domain was detected. A case-control study did not reveal a significant association between this polymorphism and schizophrenia. The results do not provide evidence that the RGS10 gene is involved in biological vulnerability to schizophrenia.

Key words G-protein-coupled receptor, GTPase-activating protein, polymorphism, regulator of G-protein signaling, RGS10, schizophrenia.

INTRODUCTION

Dysfunction of neuronal signal transduction via G-protein is thought to be involved in the pathophysiology of schizophrenia.[1] Regulator of G-protein signaling (RGS) is a protein that plays a role in modulating signaling through G-protein pathways. Over 20 mammalian subtypes of RGS proteins have been identified to date. All RGS proteins share a conserved 120-amino acid sequence termed the RGS domain. They function primarily as GTPase-activating proteins (GAP) for Gα proteins, accelerating the hydrolysis of GTP-bound-Gα proteins and shortening the duration of intracellular signaling of many G-protein-coupled receptors (GPCR), including dopamine, GABA, 5-HT, glutamate, and other neurotransmitter systems.[2] Recently, transcription of RGS4, a member of the RGS family, was found to be decreased in postmortem cerebral cortex of schizophrenia patients.[3] Among 26 single nucleotide polymorphisms (SNP) of the RGS4 gene, several in the upstream sequence and in the first intron were found to be associated with schizophrenia.[4] In addition, striatal gene expression of RGS2 and RGS4 were found to be specifically mediated by dopamine D1 and D2 receptors.[5] Thus, RGS proteins may have some potential as targets for drugs for neuropsychiatric disorders.[6]

RGS10, which belongs to the RGS family, consists of 173 amino acids and the RGS10 gene encompasses five exons spanning more than 36.5 kb of genomic DNA. RGS10 acts specifically on activated forms of the two G-proteins subunits, Gαi3 and Gαz.[7] RGS10 is highly expressed in brain regions that have been implicated in the pathophysiology of schizophrenia, such as prefrontal cortex and hippocampus.[8] In the prefrontal cortex and the hippocampus, levels of RGS10 mRNA were significantly reduced 24 h after acute and chronic electroconvulsive seizures.[8] Such seizures are known to improve psychotic symptoms. In addition, the RGS10 gene is located on chromosome 10q26.11,[9] which has been linked to schizophrenia and/or bipolar affective disorder.[10,11] Based on these findings, we hypothesized

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that some polymorphisms of the RGS10 gene which cause amino acid substitutions, if they exist, are involved in the biological susceptibility to schizophrenia through interference in the functions of this protein, such as its GAP activity. To test this hypothesis, we screened for functional polymorphisms in the coding region of the RGS10 gene and examined whether any of them were associated with schizophrenia. In addition, we assessed the frequency of another previously identified SNP in the coding region of the RGS10 gene that causes an amino acid substitution and that possibly alters the functions of this protein.

METHODS

This study was approved by the Ethics Committee of Kobe University Graduate School of Medicine. All subjects were given written informed consent to participate in this study. The study group comprised 311 unrelated Japanese patients (178 males and 133 females; mean age ± SD, 50.7 ± 17.2 years) who met the Diagnostic and Statistical Manual-IV diagnostic criteria for schizophrenia. The control subjects comprised 345 adults (205 males and 140 females; mean age ± SD, 52.0 ± 18.5 years). They were recruited from hospital staff documented to be free of psychoses, and company employees who did not manifest psychiatric problems in brief interviews by psychiatrists.

We selected 33 of the 311 schizophrenia patients at random and screened them for genetic variations in the coding region of the RGS10 gene by direct sequencing. The genomic structure of the RGS10 gene was determined from the National Center for Biotechnology Information database[12] (NT_030059 and AF045229). Genomic DNA was extracted from whole blood. All coding regions were amplified by the polymerase chain reaction (PCR). The following primer pairs for the amplification of the coding sequences were generated: pair 1, 5′-GGA AAC CAG TGG CCA TCT GT-3′ and 5′-CTC TGT GTC CTC ACA GCA CA-3′ (fragment size 285 bp, annealing temperature [Ta] 60°C); pair 2, 5′-GGC AGT GCT GAC CAT TGC AT-3′ and 5′-GAG TGC TAC GAC AGA CA-3′ (308 bp, Ta 60°C); pair 3, 5′-GGG GTT TTC GAA GAG TAA CT-3′ and 5′-GGC AAC TTC AGA ATC TAT TG-3′ (295 bp, Ta 56°C); pair 4, 5′-CCT TTC TCT TGC CAC AGC AG-3′ and 5′-GGA GTC TAA CAT CGG GTG TT-3′ (346 bp, Ta 58°C); and pair 5, 5′-GGG ATG ATT TAT GGC CTG GA-3′ and 5′-CCC ATT GAA GGG TTT TGT AC-3′ (329 bp, Ta 56°C). The sequencing reaction was performed with the ABI PRISM DNA sequencing kit (Applied Biosystems, Foster City, CA, USA). The labeled products were purified and were analyzed with an automated capillary sequencer.

In genotyping for the identified SNP Val38Met, a 308-bp fragment of the RGS10 gene was amplified by PCR with the primer pair 2. Subsequently, the PCR products were digested with the restriction enzyme *Nla*III, followed by agarose gel electrophoresis. The Met38 allele was cleaved into 168, 119 and 21 bp fragments, whereas, the Val38 allele was cleaved into 287 and 21 bp fragments.

We also genotyped another SNP, GCC to GTC (Ala88Val) in exon 4, corresponding to cDNA nucleotide position 395, which was obtained from the NCBI SNP database (ID no. rs1802228). A 346-bp fragment of the RGS10 gene was amplified using the primer pair 4. Genotyping for the Ala88Val polymorphism was performed by digestion with the restriction enzyme *Stu*I, followed by agarose gel electrophoresis.

Differences in the genotype and allele frequencies of the polymorphisms between the patients with schizophrenia and the control subjects were tested for significance using the two-sided Fisher's exacts test. Probability differences of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

One missense mutation GTG to ATG (Val38Met) was detected in exon 2 of the RGS10 gene, corresponding to cDNA nucleotide position 244. We failed to detect other missense SNP or synonymous SNP in the coding region. Table 1 shows the genotype distribution and allele frequencies of the Val38Met polymorphism in the patients with schizophrenia and the control subjects. The genotype distributions of the Val38Met polymorphism in both groups were in Hardy–Weinberg equilibrium. However, the genotype distribution and allele frequencies in the patients with schizophrenia did not differ from those in the control subjects (genotype: $\chi^2 = 1.371$, d.f. = 2, $P = 0.574$; allele: $\chi^2 = 0.060$, d.f. = 1, $P = 0.833$). The Ala88Val mutation was not found in our Japanese samples.

In this study, we detected a novel missense polymorphism in the RGS10 gene. Although it is not clear whether the Val38Met polymorphism affects the RGS10 functions, this polymorphism is located in the RGS domain which mediates the GAP activity of the RGS10 protein. Therefore, this polymorphism may change the duration of intracellular signaling of the G-protein-coupled receptors. Functional studies of this polymorphism are necessary to evaluate the enzymatic activity of RGS10.

We found no association between the Val38Met polymorphism of the RGS10 gene and schizophrenia. Our study did not provide evidence that the RGS10 gene plays a significant role in the genetic predisposi-

Table 1. Genotype distributions and allele frequencies for the Val38Met polymorphism of the RGS10 gene in patients with schizophrenia and the controls

Group	Genotype *n* (%) Val/Val	Val/Met	Met/Met	Allele *n* (%) Val	Met
Schizophrenia	300 (96.5)	11 (3.5)	0 (0.0)	611 (98.2)	11 (1.8)
Controls	335 (97.1)	9 (2.6)	1 (0.3)	679 (98.4)	11 (1.6)

tion to schizophrenia. However, the power of the association analysis was calculated at approximately 0.12.[13] We cannot exclude the possibility that our failure to find an association between the Val38Met polymorphism of the RGS10 gene and schizophrenia is due to a type II error. So, it is necessary to conduct association studies of this polymorphism with schizophrenia in large samples. Moreover, we should investigate other SNP at other sites in the RGS10 gene, such as the promoter region or intronic sequences or other flanking sequences, and make association studies with such polymorphisms to clarify whether the RGS10 gene is involved in biological vulnerability to schizophrenia. In addition, it is necessary to examine whether the Val38Met polymorphism is associated with other psychiatric disorders because the RGS10 gene is located on chromosome 10q26.11, which was confirmed to be one of the bipolar affective disorder susceptibility loci.[10]

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Association between Catechol-O-Methyltransferase Functional Polymorphism and Male Suicide Completers

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Suicide has been suggested to involve catecholaminergic dysfunction and to be related to genetics. Catechol-O-methyltransferase (COMT) 158Val/Met polymorphism (GenBank Accession No. Z26491) is a polymorphism of the gene encoding COMT, a major enzyme in catecholamine inactivation. The COMT 158Val/Met polymorphism affects COMT activity, that is, the alleles encoding Val and Met are associated with relatively high and relatively low COMT activity, respectively. In this study, we hypothesized that the COMT 158Val/Met polymorphism is associated with suicide. The study population consisted of 163 suicide completers (112 males and 51 females). We found that the genotype distribution of the COMT 158Val/Met polymorphism was significantly different between male suicide completers and male controls ($p=0.036$), while the frequency of the Val/Val genotype, a high-activity COMT genotype, was significantly less in male suicide completers than in male controls (OR: 0.52; 95% CL: 0.31–0.89; $p=0.016$). However, this was not the case in females. Our results suggest that the Val/Val genotype is a protective factor against suicide in males.

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Keywords: suicide; catecholaminergic dysfunction; COMT 158Val/Met polymorphism; association study; gender differences

INTRODUCTION

Suicide has been suggested to involve catecholaminergic dysfunction and to be related to genetics. Catecholaminergic dysfunction has been observed in suicide. For example, low concentrations of 3-methoxy-4-hydroxyphenylglycol, a metabolite of norepinephrine, and homovanillic acid, a metabolite of dopamine, were observed in the cerebrospinal fluid of suicide attempters (Lester, 1995; Roy *et al*, 1986; Jones *et al*, 1990), and high concentrations of norepinephrine and decreased α2-adrenergic binding were observed in the prefrontal cortex of suicide victims (Arango *et al*, 1993). Genetic factors in suicide have been observed in family, twin, and adoption studies, and were found to be independent of psychiatric disorders (Roy *et al*, 1997).

Catecholaminergic dysfunction in suicide appears to be related to gene polymorphisms in catecholaminergic systems. Catechol-O-methyltransferase (COMT) is a major enzyme in catecholamine inactivation. COMT has a polymorphism named COMT 158Val/Met polymorphism (GenBank Accession No. Z26491), in which Val at codon 158 is replaced with Met. The alleles encoding Val and Met are associated with relatively high and relatively low COMT activity, respectively. The Val/Val genotype leads to a level of enzymatic activity three to four times that generated by the Met/Met genotype, while the Val/Met genotype leads to an intermediate activity (Lachman *et al*, 1996).

The COMT 158Val/Met polymorphism has been shown to be associated with suicide-related disorders. For example, the Met allele (low COMT activity) was associated with aggressive behaviors or violent suicide attempts in schizophrenic patients (Strous *et al*, 1997; Lachman *et al*, 1998; Kotler *et al*, 1999; Nolan *et al*, 2000). The Met allele was also associated with depressive disorders (Ohara *et al*, 1998), although other studies did not find such an association (Frisch *et al*, 1999; Kunugi *et al*, 1997b).

We hypothesized, therefore, that the COMT 158Val/Met polymorphism is associated with suicide, independently of psychiatric disorders. To test this hypothesis, we conducted a study of the association between the COMT 158Val/Met polymorphism and suicide completers in a Japanese population.

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Table 1 Genotype and Allele Frequencies of COMT 158Val/Met Polymorphism in Suicide Completers and Controls

	Genotype frequency			Allele frequency	
	Val/Val (%)	Val/Met (%)	Met/Met (%)	Val (%)	Met (%)
Suicide completers ($n=163$)	68 (42%)	79 (48%)	16 (10%)	215 (66%)	111 (34%)
Controls ($n=169$)	90 (53%)	61 (36%)	18 (11%)	241 (71%)	97 (29%)
Male suicide completers ($n=112$)	43 (38%)	60 (54%)	9 (8%)	146 (65%)	78 (35%)
Male controls ($n=114$)	62 (54%)	42 (37%)	10 (9%)	166 (73%)	62 (27%)
Female suicide completers ($n=51$)	25 (49%)	19 (37%)	7 (14%)	69 (68%)	33 (32%)
Female controls ($n=55$)	28 (50%)	19 (35%)	8 (15%)	75 (68%)	35 (32%)

METHODS

Subjects

The study population consisted of 163 suicide completers (112 males: mean age $\pm$ SD, 48.68 $\pm$ 16.9 years; 51 females: 47.12 $\pm$ 19.8 years), who were autopsied at the Division of Legal Medicine, Kobe University Graduate School of Medicine. All subjects were ethnically Japanese. The methods of suicide were hanging (80), jumping from heights (49), drug overdose (8), drowning (8), several deep cuts (5), jumping in front of a vehicle (4), burning (3), gas poisoning (2), and other methods (4). Most (155) of the cases were classified as violent suicides according to the criteria proposed by Asberg *et al* (1976). Accurate information about the clinical backgrounds of the suicide completers could not be obtained under our ethical code for genetic studies.

Control subjects (159 males, 223 females) were recruited from the general population of Kobe city area, Japan. All were healthy and of Japanese descent and none manifested psychiatric problems in brief interviews by psychiatrists. To match the age and gender of the suicide subjects, 169 subjects (114 males: 45.33 $\pm$ 15.5 years; 55 females: 48.9 $\pm$ 18.7 years; all unrelated) were randomly selected from the above group. Informed consent was obtained from each control subject. This study was approved by the Ethical Committee for Genetic Studies of Kobe University Graduate School of Medicine.

Genotyping

Peripheral blood was drawn from suicide completers and controls, and leukocyte DNA was extracted for genotype determination. The genotypes of the COMT 158Val/Met polymorphism were determined by the method of Daniels *et al* (1996).

Statistical Analyses

The genotype distribution and Hardy-Weinberg equilibrium were tested with the χ^2 test for quality of fit. Comparisons of the genotype or allele frequencies between groups were performed with a χ^2 test. The level of significance was set at $p=0.05$.

RESULTS

Table 1 shows the genotype and allele frequencies of the COMT 158Val/Met polymorphism in suicide completers and control subjects. There is a strong trend towards deviation from Hardy-Weinberg equilibrium in the male completers ($\chi^2=3.6$; df = 1, $p=0.057$), while the genotype distributions in male/female controls and female suicide completers are in Hardy-Weinberg equilibrium. The allele frequencies in the controls were similar to those previously established for a Japanese population (Kunugi *et al*, 1997a; Ohmori *et al*, 1998; Ohara *et al*, 1998).

When the results for both genders were combined, the genotype distribution tended to be different between suicide completers and controls, although the difference was not significant ($\chi^2=5.4$; df = 2, $p=0.068$). The allele frequencies were not different between suicide completers and controls ($\chi^2=2.2$; df = 1, $p=0.14$). Similar results were obtained when only the violent suicide completers (155 of 163 subjects) were considered (data not shown).

In males, the genotype distribution was significantly different between suicide completers and controls ($\chi^2=6.7$; df = 2, $p=0.036$). The genotype distribution of male suicide completers is also significantly different from controls of both genders combined ($\chi^2=8.40$; df = 2, $p=0.015$, statistically significant tests are those having p values less than 0.017, alpha = 0.05/3). The Val/Val genotype appeared less frequently in male suicide completers than in male controls. The Odds ratio for the Val/Val genotype *vs* the other genotypes was 0.52 (95% CL: 0.31–0.89; $p=0.016$) in male suicide completers. The Val/Met genotype appeared more frequently in male suicide completers than in male controls. The Odds ratio for the Val/Met genotype *vs* the other genotypes was 1.98 (95% CL: 1.16–3.37; $p=0.012$) in male suicide completers. In allele frequencies, the Val allele tended to appear less frequently in male suicide completers than in male controls ($\chi^2=3.08$; df = 1, $p=0.080$).

Among females, no significant differences were found between suicide completers and controls in either genotype distribution ($\chi^2=0.086$; df = 2, $p=0.96$), or in allele frequencies ($\chi^2=0.007$; df = 1, $p=0.93$).

DISCUSSION

This is the first study to examine the association between the COMT 158Val/Met polymorphism and suicide completers. We found that the Val/Val genotype, a high-activity COMT genotype, appeared less frequently in male suicide completers than in male controls. Among males, the risk for suicide in the Val/Val genotype carriers was only about half that in other genotype carriers (Odds ratio 0.52). However, this was not the case in females. Consequently, our results suggest that (1) the Val/Val genotype is a factor that protects against suicide, and (2) the COMT 158Val/Met polymorphism is associated with suicide, specifically in males.

Our finding that the Val/Val genotype is a factor that protects against suicide implies that the other genotypes including the Met allele, a low-activity COMT allele, increase suicide risk. This generally supports the results of previous reports that the Met allele is associated with suicide-related disorders (Ohara *et al*, 1998; Strous *et al*, 1997; Lachman *et al*, 1998; Kotler *et al*, 1999; Nolan *et al*, 2000). In our study, the Met allele tended to appear more common in suicide completers, although it did not reach statistical significance. The higher frequency of the Met allele was mainly due to the increased Val/Met genotype rather than to an increased Met/Met genotype. Why the Met/Met genotype did not increase is not clear. One possibility is that the frequency of the Met/Met genotype in our Japanese subjects is so low that the sample size is insufficient to detect a difference of the Met/Met genotype frequencies between suicide completers and controls. The frequency of the Met allele in Japanese subjects (approximately 0.3) is lower than that in Caucasian subjects (approximately 0.5) (Palmatier *et al*, 1999). Furthermore, in another Japanese study, the Val/Met genotype appeared more frequently in schizophrenics than in the controls, while the frequency of the Met/Met genotype was not significantly different between the two groups (Ohmori *et al*, 1998).

Our finding that the COMT 158Val/Met polymorphism is associated with suicide specifically in males implies that this polymorphism affects catecholaminergic systems differently in males and females. One possible explanation for the gender-specific association is that estrogen in females modulates neurotransmission and neuronal excitability of catecholaminergic systems (Balthazart *et al*, 1996). In previous studies, the COMT 158Val/Met polymorphism has been gender-specifically associated with several neuropsychiatric disorders: obsessive–compulsive disorders (Karayiorgou *et al*, 1999), narcolepsy (Dauvilliers *et al*, 2001), and attention deficit hyperactivity disorder (Qian *et al*, 2003). Moreover, the Met allele has been associated with violent suicide attempts specifically in male schizophrenic patients (Nolan *et al*, 2000).

In our study, the genotype distribution in the male suicide completers tended to deviate from Hardy–Weinberg equilibrium. There is little possibility of genotyping error only in the male suicide completers because the genotype distributions in other groups (male/female controls and female suicide completers) are in Hardy–Weinberg equilibrium. There is also little possibility of false-positive results due to population stratification in our study because the Japanese population is considered ethnically homogeneous due to its geographical and historical isolation (Katoh *et al*, 2002).

Our research contains some limitations. First, psychiatric diagnoses were not available in this study under our ethical code for genetic studies. We cannot completely exclude the possibility that the genotype differences are secondary to the different frequencies of psychiatric disorders and not directly related to risk for suicide. Second, we did not test several SNPs and haplotypes in the COMT gene. A haplotype analysis (Shifman *et al*, 2002) could have detected a smaller effect of the COMT 158Val/Met polymorphism on suicide completers. Third, the sample size of the subjects enrolled may be insufficient. Especially, in the comparison between female suicide completers and female controls, the power of the analysis was calculated to be 0.07. Considering that the COMT 158Val/Met polymorphism might have a very small effect on female suicide completers, we cannot completely exclude the possibility that our failure to find an association between the COMT 158Val/Met polymorphism and female suicide completers is due to a type II error. A more conclusive study with a substantially larger sample size may be required. Despite these limitations, our study provides new evidence regarding a protective factor for suicide.

In conclusion, we propose that the Val/Val genotype of the COMT 158Val/Met polymorphism, a high-activity COMT genotype, is a factor that protects against suicide specifically in males in the Japanese population.

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