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No Evidence of an Association Between Tyrosine Hydroxylase Gene Polymorphisms and Suicide Victims

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Functional alternations of noradrenergic and dopaminergic neurotransmission have been implicated in suicidal behavior. A tetranucleotide repeat polymorphism in the first intron of the tyrosine hydroxylase (TH) gene, encoding a rate-limiting enzyme for the synthesis of catecholamines, is reported to have the potential to control expression of the gene and to be associated with suicidal behavior in patients with adjustment disorders. To test the hypothesis that TH gene polymorphisms are involved in suicide through an alteration of TH function, this tetranucleotide repeat polymorphism and the other two SNPs that cause a change in amino acid sequence were examined in suicide victims who completed suicide and control subjects. No significant differences in genotype distribution or allele frequencies were found between the two groups in the three polymorphisms. These findings suggest that these functional polymorphisms are not involved in a biological susceptibility to suicide.

Several lines of evidence suggest that suicide has a genetic component. Relatives of suicide victims have been reported to commit suicide more frequently than relatives of non-suicidal mentally ill probands (14,19). In addition, the concordance rate for suicide in monozygotic twins (13.2%) was found to be significantly higher than that in dizygotic twins (0.7%) (21). The rate of suicide was found to be 15-fold higher among biological relatives than among adoptive relatives of people with affective disorders (25).

Central monoamines are important modulators of mood and behavior. Neurobiological studies in suicidal behavior consistently have demonstrated a low level of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (2), showing blunted serotonergic neurotransmission in suicide. However, the association of suicide and polymorphisms on some serotonin-related genes has been controversial. A number of alterations in noradrenergic indices are also reported in suicide victims. There is evidence that the number of noradrenergic neurons in the locus coeruleus (LC) is reduced in suicide victims (2). Levels of noradrenaline appear to be lower in the brain stem of suicide victims and the number of α_2-adrenergic autoreceptor is increased (15). The tyrosine hydroxylase (TH) immunoreactivity in the LC in postmortem brains of suicide victims was reported to be reduced by Biegon et al. (4), though Zhu et al. (27) reported elevated levels of TH in LC in suicide victims with major depression. Because the noradrenergic system in the brain is thought to play a part in the stress response, it could be involved in suicide through a dysregulation of the stress response. The main metabolites of the neurotransmitter

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dopamine, homovanillic acid and 3-methoxy-4-hydroyphenylglycol (MHPG), were much higher in violent than in non-violent suicides (12). TH is a rate-limiting enzyme in adrenaline, noradrenaline and dopamine synthesis. The human TH gene is located on chromosome 11p15.5, where at least two different studies (11, 26) showed a linkage to bipolar disorder, which is known to be a high risk for suicide (6). These lines of evidence suggest that TH is involved in the biological susceptibility to suicide. Among polymorphisms in the TH gene identified so far, a penta-allelic polymorphism consisting of several numbers of tetranucleotide repeats in the first intron and three SNPs that cause the substitutions Val81Met, Leu205Pro, Val468Met may alter the functional activity of TH protein. Albanese et al. (1) reported that allelic variations of the tetranucleotide repeats were involved in quantitative and qualitative changes in the binding ZNF191, a zinc finger protein, and that the repeats may regulate transcription. Persson et al. (18) showed the 8 repeats allele was significantly more frequent in suicide attempters with adjustment disorders than in controls. To test the hypothesis that variation of TH protein function is genetically involved in suicide, we focused on functional polymorphisms in the TH gene and examined the association between suicide victims who completed suicide and these polymorphisms. The Leu205Pro polymorphism was omitted from this study because it is rare in the Japanese population (9).

MATERIALS AND METHODS

This study was approved by the Ethical Committee for Genetic Studies of Kobe University Graduate School of Medicine. The study population consisted of 189 suicide victims (127 males and 62 females; mean age ± SD, 47.9 ± 17.6 years) who completed suicide and on whom autopsies were conducted in the Department of Legal Medicine, Kobe University Graduate School of Medicine. The controls consisted of 187 unrelated volunteers (126 males and 61 females; mean age ± SD, 44.7 ± 14.9 years). All subjects were ethnically Japanese.

Peripheral blood was drawn from suicide victims and controls, and leukocyte DNA was extracted for genotype determination. The genotype of the tetranucleotide repeat polymorphism in the first intron of the TH gene was determined as follows. PCR amplification was performed with primers, 5'-ACAGGGAACACAGACTCCATG-3' and 5'-CCTTATTTCCCTCATTCATTCATTC -3', giving 60bp, 64bp, 68bp, 72bp, 76bp products. The PCR reaction mixture contained a total volume of 30 μl consisting of 50mM KCl, 1.5mM $MgCl_2$, 200 μM of each dNTP, 0.5 μM of each primer, 0.75unit of Taq DNA polymerase, and 50-100ng genomic DNA. The PCR products were resolved by electrophoresis on a 10% polyacrylamide gel and detected by EB staining.

The genotype of the Val81Met polymorphism was determined by the RT-PCR method of Kunugi et al. (9). Allelic variations of Val468Met in exon 13 were analyzed by PCR amplification using primers, 5'-AGCTATGCCTCACGCATCCA-3' and 5'-TAGCCAATGGCACTCAGCGC-3'. The PCR products were digested with the restriction enzyme HpyCH4IV and electrophoresed on a 2% agarose gel. Differences in the genotype and allele frequencies of the three TH gene polymorphisms between suicide victims and control subjects were tested for significance using the χ^2 test and the Fisher's exact test. Probability differences of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The allele and genotype frequencies of the tetranucleotide repeat polymorphism were not significantly different between suicide victims and control subjects ($\chi^2=3.86$, $p=0.43$ and

Table 1. Genotype and allele counts (and frequencies) of tyrosine hydroxylase tetranucleotide repeat polymorphism in suicide victims and controls

	Male		Female		All Subjects	
	Suicide victims	Controls	Suicide victims	Controls	Suicide victims	Controls
Genotypes						
TH10TH10	1 (0.8%)	1 (0.9%)	0	0	1 (0.6%)	1 (0.7%)
TH10TH9	7 (5.8%)	3 (2.8%)	2 (3.5%)	3 (7.0%)	9 (5.1%)	6 (4.0%)
TH10TH8	0	2 (1.9%)	1 (1.8%)	1 (2.3%)	1 (0.6%)	3 (2.0%)
TH10TH7	1 (0.8%)	6 (5.6%)	3 (5.3%)	0	4 (2.2%)	6 (4.0%)
TH10TH6	1 (0.8%)	3 (2.8%)	0	3 (7.0%)	1 (0.6%)	6 (4.0%)
TH9TH9	17 (14.0%)	19 (17.8%)	6 (10.5%)	7 (16.3%)	23 (12.9%)	26 (17.3%)
TH9TH8	7 (5.8%)	3 (2.8%)	2 (3.5%)	0	9 (5.1%)	3 (2.0%)
TH9TH7	29 (24.0%)	20 (18.7%)	17 (29.8%)	10 (23.3%)	46 (25.8%)	30 (20.0%)
TH9TH6	26 (21.5%)	18 (16.8%)	12 (21.1%)	7 (16.3%)	38 (21.3%)	25 (16.7%)
TH8TH8	0	0	0	0	0	0
TH8TH7	5 (4.1%)	5 (4.7%)	2 (3.5%)	1 (2.3%)	7 (3.9%)	6 (4.0%)
TH8TH6	1 (0.8%)	3 (2.8%)	0	3 (7.0%)	1 (0.6%)	6 (4.0%)
TH7TH7	10 (8.3%)	6 (5.6%)	6 (10.5%)	2 (4.7%)	16 (9.0%)	8 (5.3%)
TH7TH6	11 (9.1%)	16 (15.0%)	4 (7.0%)	4 (9.3%)	15 (8.4%)	20 (13.3%)
TH6TH6	5 (4.1%)	2 (1.9%)	2 (3.5%)	2 (4.7%)	7 (3.9%)	4 (2.7%)
	X^2=16.4, p=0.23		X^2=15.1, p=0.24		X^2=20.4, p=0.085	
Alleles						
TH10	11 (4.5%)	16 (7.4%)	6 (5.3%)	7 (8.1%)	17 (4.7%)	23 (7.7%)
TH9	103 (42.6%)	82 (38.3%)	45 (39.4%)	34 (39.5%)	148 (41.6%)	116 (38.7%)
TH8	13 (5.4%)	13 (6.0%)	5 (4.4%)	5 (5.8%)	18 (5.1%)	18 (6.0%)
TH7	66 (27.2%)	59 (27.6%)	38 (33.3%)	19 (22.0%)	104 (29.2%)	78 (26.0%)
TH6	49 (20.2%)	44 (20.6%)	20 (17.5%)	21 (24.4%)	69 (19.4%)	65 (21.7%)
	X^2=2.26, p=0.69		X^2=4.13, p=0.39		X^2=3.86, p=0.43	

χ^2=20.4, p=0.085, respectively) (Table 1). Although the 8 repeats allele was found to be more frequent in suicide attempters with adjustment disorders 18), we didn't find a significant difference of the 8 repeats allele frequency between suicide victims and controls (χ^2=0.26, p=0.60). Genotypes and alleles distribution of the two SNPs which result in amino acid sequence change are shown in the table 2. The differences in the Val81Met alleles (χ^2=1.43, p=0.23) or genotypes frequencies (χ^2=1.55, p=0.46) between suicide victims and control subjects were not significant. The Val allele frequency in the Val468Met was found only 0.9% (4/458) in all the samples. We also found no significant difference in the distributions of the Val468Met alleles (p=0.36) or genotypes (p=0.36) in the statistical analysis using the Fisher's exact test. The distributions of all three polymorphisms are in Hardy-Weinberg equilibrium. We also found no significant difference of the distribution of these polymorphisms between suicide victims and controls in male samples or in female samples (Tables 1, 2). Haplotype analysis of these polymorphisms also shows no significant difference between suicide victims and controls (data not shown). These findings indicate that it is unlikely that these functional polymorphisms in the TH gene are involved in suicide in the Japanese population.

To our knowledge, this is the first association study between suicide victims who complete suicide and TH functional polymorphisms. The discrepancy between our results and the precedent study (18) that reported suicide attempters carried the 8 repeats allele significantly more frequently, might be caused by the difference of the examined samples between suicide completers and suicide attempters. On the other hand, numerous studies have found an association between TH gene polymorphisms and psychiatric disorders. Because psychiatric disorders such as mood disorder and schizophrenia are a high risk for suicide, several candidate genes in suicide might be shared with such disorders. The polymorphic repeat of a tetranuculeotide in the first intron of TH gene was reported to be associated with bipolar affective disorder by Leboyer (10) in 1990. However, most studies since then have found no such association (5, 8, 17, 23). TH gene polymorphisms were also found to be associated with schizophrenia (13, 24), although some subsequent studies failed to find a significant association (7, 16, 20). According to the ethical code of this

Table 2. Genotype and Allele counts (and frequencies) of Tyrosine Hydroxylase Val81Met polymorphisms in suicide victims and controls

	Male		Female		All Subjects	
	Suicide victims	Controls	Suicide victims	Controls	Suicide victims	Controls
Genotypes						
Met/Met	61 (50.0%)	54 (42.9%)	30 (50.8%)	26 (42.6%)	91 (50.3%)	80 (42.8%)
Val/Met	52 (42.6%)	62 (49.2%)	25 (42.4%)	26 (42.6%)	77 (42.5%)	88 (47.1%)
Val/Val	9 (7.3%)	10 (7.9%)	4 (6.8%)	9 (14.8%)	13 (7.1%)	19 (10.2%)
	χ^2=1.29, p=0.52		χ^2=2.20, p=0.33		χ^2=1.55, p=0.46	
Alleles						
Met	174 (71.3%)	170 (67.5%)	85 (72.0%)	78 (63.9%)	259 (71.5%)	248 (66.3%)
Val	70 (28.7%)	82 (32.5%)	33 (28.0%)	44 (36.1%)	103 (28.5%)	126 (33.7%)
	χ^2=0.87, p=0.35		χ^2=1.81, p=0.18		χ^2=1.43, p=0.23	

study, the samples became anonymous when examined and we could not obtain their clinical information retrospectively. Schulsinger et al. (22) proposed that some people have a genetic predisposition for suicide that is independent of, or additive to, the major psychiatric disorders. If this is the case, then a lack of information on the clinical diagnosis of the subjects should not be a disadvantage in investigating candidate genes in suicide. Although small effects may have escaped detection in the present study, it seems very unlikely that the TH gene mutation has an effect on human suicidal behavior.

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