Association between suicide attempt and a tri-allelic functional polymorphism in serotonin transporter gene promoter in Chinese patients with schizophrenia

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\textbf{A B S T R A C T}

Mounting evidence supports the association between a polymorphism in the serotonin transporter gene promoter region (5-HTTLPR) and suicidal behaviour. Recently, a novel variant of the 5-HTTLPR L allele was identified. The previously unknown L\textsubscript{2} allele produced similar levels of gene expression to the S allele and might have been misclassified as a “high-expression” allele in previous association studies. In this study, we aimed to compare the genotype distribution of the tri-allelic 5-HTTLPR polymorphism in 168 Chinese patients with schizophrenia, including 60 suicide attempters and 108 non-suicide attempters. In our analysis, which used the L\textsubscript{4} dominant model, it was found that the L\textsubscript{4} allele carriers were significantly more likely to have attempted suicide ($p = 0.035$). Further analysis showed this association existed only in male patients ($p = 0.012$). A similar association between the L\textsubscript{4} allele and violent suicide attempt was also found ($p = 0.028$). In addition, logistic regression confirmed our findings that male L\textsubscript{4} allele carriers were at a higher risk of suicide, although the lack of a significant association in females may reflect insufficient power due to small sample size. However, no association was found when we examined the traditional bi-allelic 5-HTTLPR. These findings differ from those reported in Caucasian subjects, where no associations have been reported. Different genetic backgrounds may give rise to different allelic distribution, causing differential effects on the expression of endophenotypes of suicide behaviours. Although the potential influence of multiple comparisons might weaken our findings, our study provides preliminary evidence for a potentially gender-specific role of a “high-expression” 5-HTTLPR polymorphism in susceptibility to suicide in Chinese patients with schizophrenia.

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Suicide is one of the most serious health problems in the world. The World Health Organization estimated that suicide accounts for almost 2% of all deaths in the world [28]. Reported rates of suicide attempts in patients with schizophrenia vary from 25 to 50%, and 10% of them eventually commit suicide [25]. The extant adoption, twin and family studies have shown that suicidal behaviour is both familial and heritable [6]. It is estimated that genetic factors accounted for 30–50% of the variance in suicidal thoughts and behaviour [34], and about 43% of the variance in completed suicide [24]. Numerous attempts have been made to elucidate the genetic contribution to vulnerability towards suicidal behaviour.

Since the seminal finding of Asberg et al. [1], which showed that a low cerebrospinal fluid 5-Hydroxyindoleacetic acid (5-HIAA) concentration was related to the incidence of violent suicidal acts, serotonergic dysfunction has been thought to play an important role in suicidal behaviour. Numerous studies have been conducted to investigate how genetic variants in the serotonergic system may confer susceptibility to suicide (see review [4]). One of the candidate genes thought to be linked to suicidal behaviour is the gene (SLC6A4) coding for the serotonin transporter (5-HTT). The human 5-HTT gene, located in chromosome 17q11.1-q12, has a common polymorphism (5-HTTLPR) in the 5'-regulatory region. The polymorphism is a 44 base-pair deletion/insertion, which results in either a short (S) or long (L) allele [17]. The presence of the S allele...
Briefly, polymorphism was associated with reduced transcriptional activity and lower levels of serotonin uptake in transformed lymphoblastoma cells [21]. Studies exploring the relationship between 5-HTTLPR and suicide have reported inconsistent results. Some studies have reported an association between the S allele and suicide [10,30], while others have found that the S allele is associated with violent suicide attempts [3,5], and suicide with high medical damage [36]. Another study reported a significantly higher frequency of the L allele in depressed suicide victims [12]. Other studies found no differences in the 5-HTTLPR genotype or allele frequency between suicide attempters and non-attempters in psychiatric patient populations [9,15,32].

In past few years, a functional allele (rs25531) with an A to G variation in the L allele of the 5-HTTLPR was detected [27]. The Long G (Lc, GenBank Accession number AB031254) and the Long A (La, AB031251) alleles were found to be functionally distinct. The La allele was associated with high levels of 5-HT mRNA expression, but the expression level of the Lc allele was similar to that of the S allele. These three alleles (S, Lc, La) appear to act co-dominantly [19]. Because the Lc allele has decreased transcriptional expression, which is nearly equivalent to the S allele, it is possible that the inconsistent results of earlier association studies may be due to inclusion of subjects with the Lc allele, which was classified as a “high-expression” variant.

The goals of this study are twofold: (1) to investigate the relationship between the tri-allelic 5-HTTLPR and suicidal behaviour among Chinese patients with schizophrenia; and (2) to examine if use of violent methods in suicide attempts is influenced by the polymorphism.

In this study, patients were consecutively recruited from three different hospitals in southern Taiwan. All were diagnosed as schizophrenia according to DSM-IV criteria using the Structured Clinical Interview for DSM-IV (SCID-I), conducted by a psychiatrist. Their ages varied between 20 and 65 years, and all had had the illness for at least one year. Those patients who had documented history of illicit drug or alcohol dependence, mood disorders, mental retardation, or dementia were excluded from our study. Their suicide history, including the number of suicide attempts, methods and consequences of suicide, and their family history of suicide were also examined. The methods of suicide were classified as violent and non-violent suicide attempt by Asberg’s criteria [1]. Briefly, hanging, using firearm or knives, jumping from heights, and crashing with motor vehicles were classified as violent attempts, while drug overdose and gas inhalation were considered to be non-violent suicide attempts. Healthy controls, whose age ranged from 35 to 70 years, were consecutively recruited from the general health examination centre at Kaohsiung Chang Gung Memorial Hospital. An older age range of healthy controls than patients was set so as to reduce the risk of misclassification of psychiatric disorders. All of the controls were screened using the Chinese Health Questionnaire [8] and personal interview. Subjects were excluded if they were scored 3 points or above in the questionnaire, or had a history of psychiatric disorders or suicide attempts. To avoid population stratification, both the recruited patients and controls were questioned to ascertain whether they were Han Chinese, which accounts for 98% population in Taiwan. This study was approved by the institutional review board. Informed consent was obtained from all subjects after the research purpose and procedure were explained.

Genomic DNA was extracted from peripheral leukocytes by using a commercial kit (Qiagen, Valencia, CA). The genotyping method was based on that used in a previous report [19]. The promoter region of the 5-HT gene which contains the bi-allelic polymorphism was then amplified by polymerase chain reaction (PCR) using the primers: 5′-GCC GTT GCC GCT CTG AAT GC-3′ and 5′-GAG GGA CTG AGC TGG ACA ACC AC-3′. The amplified product was next digested with MspI restriction enzyme (New England Biolabs, Ipswich, MA) which allows for the detection of the A/G (rs25531) variant present in the L allele and can identify the tri-allelic polymorphisms as it resulted in 342 bp fragments for the La allele, 168 and 174 bp fragments for the Lc allele, and 484 bp fragments for the S allele, respectively. The digestion products were then visualized by 2% agarose gel electrophoresis that was stained with ethidium bromide under UV light. We re-genotype 20% of our samples with random selection for quality control and no discrepancy was observed.

Statistical analyses were carried out by using the Statistical Package for Social Science (SPSS) software, version 15.0 for Windows (SPSS for Windows 15.0, SPSS, Chicago, IL, USA). The functionally equivalent Lc and S alleles were grouped as the lower expressing alleles (S) and next compared with the higher expressing La allele (L). Hardy-Weinberg equilibrium for genotype frequencies in suicide attempters and non-attempters were calculated using Chi-square tests. Chi-square tests for independence assessed the genotypic frequencies between the groups, unlike Fisher’s exact test was indicated. The relationship between continuous variables and suicide attempt were examined using Student’s t-tests. Finally, where the univariate analysis had revealed a significant effect of genotype specific to males, logistic regression explored the interaction between 5-HTTLPR and gender. We proposed three logistic regression models: (1) a full model for each marker including genotype, gender, and genotype by gender interaction; (2) a model without an interaction term including genotype and gender, and (3) a model stratified by gender using only genotype. These models were first fitted using the tri-allelic polymorphic genotype, and then using the bi-allelic genotype to compare model fit. Post hoc power was estimated for comparing two sample proportions, given the current sample sizes with a type I error at 0.05, and was calculated by using G’power 3 [13]. All tests were two-tailed and the level of significance was set at p < 0.05.

A total of 302 healthy controls (196 male and 106 female) and 168 unrelated schizophrenia patients including 60 suicide attempters (35 male and 25 female) and 108 non-suicide attempters (70 male and 38 female) were recruited to this study. Compared with healthy controls, our schizophrenia subjects were younger (controls: 50.1 years (SD = 8.6) versus patients: 38.3 years (SD = 9.9); t = 13.6, p < 0.001). There was no statistically significant difference between schizophrenia subjects and healthy controls in novel tri-allelic 5-HTTLPR genotype (ζ² = 0.63, df = 2, p = 0.73), suggesting that 5-HTTLPR is unlikely to be associated with schizophrenia.

There were no differences between suicide attempters and non-suicide attempters in age (37.4 years (SD = 9.6) versus 38.7 years (SD = 10.1); t = −0.83, p = 0.41), duration of illness (23.5 years (SD = 8.4) versus 24.55 years (SD = 7.3); t = −0.89, p = 0.38) and family history of suicide (ζ² = 0.02, df = 1, p = 0.90). There was also no difference in patients’ demographic characteristics including age, duration of illness and family history of suicide in each genotypic group whether using bi-allelic or tri-allelic classification (data not shown). The genotypic distribution of 5-HTTLPR for both the non-suicide attempters (ζ² = 3.09, df = 1, p = 0.08) and suicide attempters (ζ² = 0.53, df = 1, p = 0.47) among patients with schizophrenia was in Hardy-Weinberg equilibrium.

We grouped the low-expressing La allele and the S allele together and the new group was designated L’. Carriers of the high-expressing La allele were designated L. This resulted in a significant association between the novel tri-allelic 5-HTTLPR and history of suicide attempt (ζ² = 6.69, df = 2, p = 0.035) (Table 1). Further stratified analysis revealed that there was a gender-specific association between the novel tri-allelic 5-HTTLPR genotype and suicide attempt in male (ζ² = 8.83, df = 2, p = 0.012) but not female patients (ζ² = 0.77, df = 1, Fisher’s exact test p = 0.460) (Table 1). However,
lack of an association in female group may well be attributed to lack of power due to small sample size.

Due to the small sample size in the L’ group, both the L’ and L’S groups were pooled together as L’ carriers (dominant mode). Again, we found a significant association of the L’ carrier and suicide attempt (data not shown). When analysing the data using traditional bi-allelic genotype classification, however, our study found that 5-HTTLPR genotype did not differ between schizophrenic patients who had attempted suicide and schizophrenic non-suicide attempters ($\chi^2 = 2.33, df = 2, p = 0.312$).

Logistic regression was used to explore the relationship between 5-HTTLPR polymorphism and gender in conferring risk of suicide attempts. Comparing three different logistic regressions models, we found no evidence for an interaction between genetic polymorphisms and gender in conferring risk ($p > 0.05$). The regression model without the interaction term (Akaike’s Information Criterion, AIC = 219.95) fitted better across all genotype groups, especially the L’S group, compared to full model (Table 2) and remained significant using an L’ dominant model (L’ carriers: $b = 0.77$, Wald $\chi^2(1) = 4.66, p = 0.03$, OR = 2.17 (1.07–4.37); gender: $b = -0.30$, Wald $\chi^2(1) = 0.80, p = 0.37$, OR = 0.74 (0.38–1.43)). Further analysing data stratified by gender, the model with L’ carriers (AIC = 131.21) was the best fitting regression model and was, again, more appropriate in male (L’ carrier: $b = 1.10$, Wald $\chi^2(1) = 5.86, p = 0.015$, OR = 3.00 (1.23–7.30)) but not in female.

In examining the association between the 5-HTTLPR and the methods of suicide attempt, we found that violent suicide attempt was significantly associated with the novel tri-allelic ($\chi^2 = 7.13, df = 2, p = 0.028$) but not with the traditional bi-allelic 5-HTTLPR polymorphism ($\chi^2 = 2.41, df = 2, p = 0.300$). However, no specific gender effect was noted (all $p > 0.05$ in both genders either using bi-allelic or bi-allelic analysis). However, when using the L’ dominant mode, only a marginal association was noted between violent and the polymorphism. Furthermore, neither the tri-allelic nor bi-allelic 5-HTTLPR was found to be associated with repetition of suicide attempts (multiple: $\chi^2 = 0.98, df = 2, p = 0.61$ versus single: $\chi^2 = 0.24, df = 2, p = 0.89$).

Many studies have investigated the association between 5-HTTLPR and suicide, but results of these studies have been inconsistent [23]. This may be due to analyses failing to take into account a common A to G substitution on the L allele, which renders it a low expressing variant. In our study, when using the traditional bi-allelic genotyping analysis, no association was found between 5-HTTLPR and suicide attempts. This finding is consistent with previous studies that used Chinese subjects with schizophrenia [9] and psychiatric disorders [32,38]. On the other hand, when analysing the novel tri-allelic genotyping, a significant difference in 5-HTTLPR genotype frequencies between the suicide attempters and non-attempters was found. However, studies that have used Caucasian subjects have failed to find an association between the tri-allelic 5-HTTLPR and suicidal behaviour in patients with schizophrenia (cases number ranged between 92 and 150, and controls number ranged between 198 and 200) [11,35] or depression (94 cases and 94 controls) [31]. Such a discrepancy may arise from the different frequency of the L allele and even Lc allele between Caucasian and Chinese populations. Ethnic differences in allelic frequencies for the long allele have been noted, ranging from 0.29 in the Chinese to 0.60 among Caucasians [16,17]. Furthermore, in terms of A/G variant in the L allele, our study indicates that the frequency of Lc allele is approximately 0.51 in Chinese subjects, whereas the allelic frequency of Lc is 0.09–0.14 in Caucasians and 0.24 in African Americans [31,37]. As such, nearly half of the L alleles were mis-classified as high expressing using the traditional bi-allelic approach. Our study found that 25.6% of our subjects were classified differently based on traditional bi-allelic from novel tri-allelic functional model, thus resulting in the significant findings using
the novel tri-allelic approach. These results suggest that ethnicity has a differential impact on suicide behaviours through its impact on the distribution of 5-HTTLPR genotypes. Further stratification of our subjects by gender revealed that the association exists solely in male patients, which could possibly suggest a gender-specific association between 5-HTTLPR and suicide attempt. Males with schizophrenia have a higher risk of suicide. Suicide accounts for 50% of deaths among first-episode schizophrenic male patients and 35% of schizophrenic female patients [26]. Sexual dimorphisms in the serotonin system had been recognised for many years [20], but the genetic effects of the 5-HTTLPR on gender difference in psychiatric disorders or suicidal behaviour are largely unknown and findings have been inconsistent. One study suggested that female-specific association exists between the 5-HTTLPR and suicide attempts [2], but another study indicated that there is male-specific association between 5-HTTLPR S-allele and suicide attempts in subjects with alcohol dependence [22]. Two studies [7,33] have suggested that the effects of the 5-HTTLPR on depression were moderated by psychosocial stressors and gender. Males and females carrying the S allele have been found to develop different responses to environmental stress [33]; while females tend to develop depressive symptoms, males seem to be protected from depression. Brummett et al. found that females carrying the S allele, combined with life stress, was associated with higher depression scores; whereas males carrying the L allele, combined with a stressor, was associated with higher depression scores [7]. Although our study did not include psychosocial measurements, our results provide preliminary evidence showing male-specific role of the “high-expression” allele in suicide attempts. The lack of a significant association in females may well reflect a lack of statistical power as power analysis indicated that we had only 28% power to detect a small effect in females. Therefore, we must point out the lack of association in female patients might only reflect insufficient power to detect small effects. Further studies are needed to investigate gender-specific gene-environment interactions in suicidal behaviour.

The current study is the first to report an association between novel tri-allelic 5-HTTLPR and suicide attempts in Han Chinese patients with schizophrenia. However, this study has several limitations. First, the sample size is small in our study, which may increase the type II error. A post hoc power calculation (with a modest effect size of 0.2, or error probability of 0.05, total sample of 168 subjects, and 2 degrees of freedom) revealed 64% power to detect an effect in our study. Therefore, the study had limited power to detect small effect sizes. Second, psychosocial stressors were not assessed in this study, limiting our exploration of gene-environment interactions. Third, the present study is retrospective in nature, and the influence of recall bias could not be ruled out. Fourth, another functional polymorphism, a 17-bp variable number of tandem repeats (5′Stin2 VNTR) was not taken into account in this analysis. Previous studies have reported inconsistent findings as to the role of 5′Stin2 in suicide attempt, either using a single functional polymorphism analysis or haplotype analysis combined with 5-HTTLPR [14,18,29]. It will be important to include this polymorphism for haplotype analyses in the future studies. Lastly, exploring the effect of the polymorphism on different outcomes without correcting for multiple comparisons raises the probability of a type I error, though Bonferroni correction would have been inappropriate as these are not independent outcomes. Instead, we performed three different logistic regression models to correct it. As such, our results should be considered preliminary and await replication.

In summary, our study found preliminary evidence for an association between the L4 allele of novel tri-allelic 5-HTTLPR polymorphism and history of both suicide attempts, and use of violent methods for suicide attempts in Chinese patients with schizophrenia.

Males carrying the L4 allele were at a higher risk of suicide attempts than females, but no gender effect was found for violent suicide attempts. Our sample size is relatively small, and the exploration of the impact of 5-HTTLPR on different suicide related outcomes raised the possibility of a Type I error, so the results should be regarded as preliminary before being confirmed by further large-scale studies.

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References


Table 2

Risk factors for suicide attempts in patients with schizophrenia: logistic regression analysis using an L′ dominant model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>df</th>
<th>p</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-0.623</td>
<td>0.279</td>
<td>5.003</td>
<td>1</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>L′ dominant</td>
<td>0.773</td>
<td>0.358</td>
<td>4.664</td>
<td>1</td>
<td>0.03</td>
<td>2.2 (1.07–4.37)</td>
</tr>
<tr>
<td>S′</td>
<td>-0.301</td>
<td>0.336</td>
<td>0.802</td>
<td>1</td>
<td>0.37</td>
<td>0.74 (0.38–1.43)</td>
</tr>
</tbody>
</table>

| Abbreviations: B, intercept in logistic regression equation; S.E., standard error; Wald, Wald chi-square test; df, degrees of freedom; OR, odds ratio; CI, confidence interval. |
|---|---|---|---|---|---|---|
| **L′** | S′ | L′ vs. S′ gender (L′ = L4L4, S′ = L4L5 + L5L5; S′ = L5L5 + L4S′ + SS). |
| **Reference group female.** |

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