Research Report

Association of brain-derived neurotrophic factor (Val66Met) genetic polymorphism with methamphetamine dependence in a Malaysian population

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ABSTRACT

Methamphetamine is a highly addictive psychostimulant that has surged in popularity worldwide in the last decade. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophic factor family, is widely expressed in the adult mammalian brain and plays an important role in the long-term survival, differentiation, and outgrowth of neurons. Previous studies suggested that the BDNF gene may be involved in the mechanisms underlying substance dependence. This study investigated the association of the BDNF gene Val66Met polymorphism with methamphetamine dependence and with psychosis in a Malaysian population with different ethnicities. The BDNF Val66Met polymorphism was genotyped by PCR-RFLP in 186 male methamphetamine-dependent subjects and in 154 male controls of four different ethnicities, namely, Malay, Chinese, Kadazan-Dusun, and Bajau. Our results showed that the distribution of the BDNF Val66Met genotype in Chinese subjects with methamphetamine dependence (OR=2.6, p=0.015) and methamphetamine psychosis (OR=0.2, p=0.034) were significant compared with controls. The frequency of the 66Val allele in methamphetamine-dependent subjects was higher than that in the control group, suggesting that the 66Val carriers are more susceptible to methamphetamine dependence. However, 66Val allele frequency in other ethnicities was not significantly different from the controls. The results of the study also showed that in the Chinese methamphetamine-dependent subjects, there was a difference in allele frequency when comparing those who developed psychosis and those who did not. Our findings suggest that the BDNF Val66Met polymorphism may contribute to methamphetamine dependence and psychosis in the Chinese population but not in other Malaysian ethnicities.

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Abbreviations: BDNF, brain-derived neurotrophic factor; DRD3, dopamine D3 receptor; Val, valine; Met, methionine; OR, odds ratio; CI, confidence interval; SD, standard deviation; N and n, number

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1. Introduction

Substance dependence remains a worldwide problem, and its negative impact on society is increasing. It is a chronic relapsing disorder in which compulsive drug-seeking and drug-taking behaviour persists despite serious negative consequences. The past decade has seen a remarkable increase in the popularity of methamphetamine within East Asia and the Pacific region. The United Nations Office on Drugs and Crime (UNODC) estimated that in 2005, 26 million people in the world used methamphetamine, 11 million used opiates, and 14 million used cocaine (United Nations Information Service (UNIS), 2005). It was also reported that 60% of methamphetamine users live in Asia. The impact of the spread of methamphetamine use, with its serious behavioural, medical, and psychiatric consequences, is being felt at the individual, familial, community, and societal levels, placing a tremendous strain on the medical, public health, and criminal justice systems. In Malaysia, the National Anti-Drug Agency (2008) identified 8870 addicts (January to August 2008), of whom 1126 were dependent on methamphetamine.

The Malaysian population is made up of various ethnic groups. Geographically, Malaysia is separated by the South China Sea into two regions, Peninsular Malaysia (also known as West Malaysia) and Malaysian Borneo (also known as East Malaysia), the latter being made up of two states, Sabah and Sarawak. According to the Yearbook of Statistics, Sabah (2001) from the Department of Statistics Malaysia (2000), the ethnic composition of West Malaysia and East Malaysia is very different, whereby the Malays (54% of the total Malaysian population); 11.5% of the Sabah population) and Chinese (25% of the total Malaysian population; 13.0% of the Sabah population) make up the majority ethnic group in West Malaysia, while in East Malaysia, the Kadazan-Dusun (2.5% of the total Malaysian population; 18.4% of the Sabah population) and Bajau (1.5% of the total Malaysian population; 13.5% of the Sabah population) make up the majority ethnic group. The subjects of this study were taken from a Rehabilitation Centre that is located in Sabah in which the majority of the population is of the Kadazan-Dusun and Bajau ethnic groups, besides the presence of other ethnic groups including the Chinese and Malays.

Brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor-related family of neurophins, is widely expressed in the adult mammalian brain. Evidence indicates that BDNF may be involved in the mechanisms underlying substance abuse (Hofer et al., 1990). BDNF plays an important role in the neurodevelopment of dopaminergic (DA)-related systems. This protein interacts with the meso-limbic DA systems that are involved in the therapeutic response to substance abuse, and it subsequently promotes and maintains dopamine D3 receptor (DRD3) expression (Krebs et al., 2000).

Methamphetamine is a drug that easily induces drug conditioning and elevated BDNF mRNA. BDNF expression has been found to increase acutely after drug abuse, leading to subsequent long-lasting elevation of DRD3 in the nucleus accumbens, which may facilitate response to drug-associated stimuli and finally induce addictive disorders (Le Foll et al., 2005). Genomic scans have demonstrated that the BDNF gene is associated with vulnerability to drug abuse (Itoh et al., 2005). Recent pharmacogenomic studies have demonstrated the involvement of some single nucleotide polymorphisms in the response to drugs. The susceptible single nucleotide polymorphisms in some genes may contribute to addiction vulnerability in several ways, including changing the structure or function of specific proteins and altering the expression of brain circuit proteins during development or in adulthood. The altered brain circuits could change the responsiveness of the individual to initial drug exposure or to adaptations that occur in the brain after repeated drug exposure. However, environmental factors are also important and could affect addiction vulnerability by influencing the same neural circuits (Nestler, 2000).

Recent studies have demonstrated that the BDNF gene is associated with vulnerability to drug abuse (Itoh et al., 2005). Furthermore, Flanagan et al. (2006) reported that BDNF has been implicated in the behavioural response to psychomotor stimulants and that it potentiates neurotransmitters, which are strongly linked to addiction. They suggested that this gene is a logical candidate gene for the study of addiction. Data derived from animal studies have demonstrated that BDNF modulates dopaminergic and serotonergic functions that are strongly linked to substance abuse (Dluzen et al., 1999).

BDNF, located on chromosome 11p13, contains a common and functional single nucleotide polymorphism, rs6265 (Val66Met), at codon 66. This G196A polymorphism results in a valine (Val) to methionine (Met) substitution in the prodomain, which affects intracellular trafficking and activity-dependent secretion of BDNF (Li et al., 2005). A previous study reported that the valine (196G) allele is more commonly methamphetamine dependent and suggested that the GG genotype is a risk factor for substance abuse, related to late onset of substance abuse (Cheng et al., 2005).

According to Haile et al. (2009), this BDNF polymorphism is not only linked to methamphetamine dependence, but also to the development of methamphetamine psychosis. A study on the human BDNF gene has demonstrated that the Val66Met BDNF gene polymorphism is associated with methamphetamine dependence in methamphetamine-dependent Taiwanese subjects (p = 0.046), suggesting that homozygous carriers of the 196G allele are more susceptible to methamphetamine abuse (Cheng et al., 2005). This finding suggested that the Val66Met BDNF polymorphism may confer risk for substance abuse. In the present study, we examined the association of Val66Met BDNF with methamphetamine dependence and the occurrence of psychosis in a methamphetamine-dependent Malaysian male population. Besides that, the age of onset of methamphetamine dependence was also examined in the study.

2. Results

Results from RFLP indicated that three variants exist in the digestion product: homozygous 66Met (GG), heterozygous (GA), and homozygous 66Val (AA) (Fig. 1). The presence of 168-bp and 75-bp bands indicated the existence of the A (66Met) allele; the presence of a 243-bp band indicated the existence of the G (66Val) allele; and the presence of 75-bp, 168-bp, and 243-bp...
With regard to the occurrence of methamphetamine psychosis, no significant difference in either genotype frequency ($p=0.850$) or allele frequency ($p=0.889$) was seen when comparing methamphetamine-dependent subjects who experience psychosis with those who do not. However, examination of the data according to the ethnicities revealed that there was a significant difference in allele frequency in the Chinese methamphetamine-dependent subjects ($p=0.034$) (Table 2).

The frequency of carrying the A allele in Chinese methamphetamine-dependent subjects with psychosis was higher than in the methamphetamine-dependent subjects without psychosis. Comparison of the $66\text{Val}$ allele frequency with $66\text{Met}$ allele frequency revealed a significant difference in odds ratio (OR: $0.2$, 95% CI $0.07-0.6$, $p=0.034$). The results showed no significant difference in genotype and allele frequency between methamphetamine-dependent subjects with and without psychosis among the other races studied.

Moreover, the effect of the BDNF $Val66\text{Met}$ genetic polymorphism on the age of onset of methamphetamine abuse was also analyzed in 187 methamphetamine-dependent subjects. The mean age of onset for the methamphetamine-dependent groups was $24.8\pm9.0$ years old for BDNF $66\text{Val}/66\text{Val}$ ($n=57$), $23.2\pm9.4$ years old for BDNF $66\text{Val}/66\text{Met}$ ($n=99$), and $23.4\pm5.9$ years old for BDNF $66\text{Met}/66\text{Met}$ ($n=31$). For the age of onset among the Chinese dependent group, the mean age was $30.3\pm8.5$ years old for BDNF $66\text{Val}/66\text{Val}$ ($n=10$), $31.1 \pm 11.493$ years old for BDNF $66\text{Val}/66\text{Met}$ ($n=12$), and $24.0 \pm 11.314$ years old for BDNF $66\text{Met}/66\text{Met}$ ($n=2$). The age of onset of the methamphetamine-dependent subjects neither in overall dependent subjects ($p=0.539$) nor in Chinese ($p=0.593$), Malay ($p=0.822$), and Kadazan-Dusun ($p=0.762$) and Bajau ($p=0.371$) dependent subjects was not significant.

### 3. Discussion

Our findings failed to prove an association between BDNF $Val66\text{Met}$ genotype or allele frequency and methamphetamine dependence in the overall Malaysian subjects studied. In a recent study in 189 methamphetamine abusers from a Japanese population (79.4% male and 20.6% female), BDNF $Val66\text{Met}$ polymorphism was not associated with methamphetamine

### Table 1 - Genotype and allelic frequencies of the BDNF Val66Met polymorphism in male controls and male methamphetamine-dependent subjects.

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Participant</th>
<th>Genotype</th>
<th>Allele frequency</th>
<th>OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td>Participant</td>
<td>G/G</td>
<td>G/A</td>
<td>A/A</td>
</tr>
<tr>
<td>Malay</td>
<td>Case subject</td>
<td>20 (0.339)</td>
<td>33 (0.559)</td>
<td>6 (0.102)</td>
</tr>
<tr>
<td>Malay</td>
<td>Control</td>
<td>16 (0.314)</td>
<td>25 (0.490)</td>
<td>10 (0.196)</td>
</tr>
<tr>
<td>Chinese</td>
<td>Case subject</td>
<td>10 (0.417)</td>
<td>12 (0.500)</td>
<td>2 (0.083)</td>
</tr>
<tr>
<td>Chinese</td>
<td>Control</td>
<td>6 (0.133)</td>
<td>27 (0.600)</td>
<td>12 (0.267)</td>
</tr>
<tr>
<td>Kadazan-Dusun</td>
<td>Case subject</td>
<td>13 (0.260)</td>
<td>28 (0.560)</td>
<td>9 (0.180)</td>
</tr>
<tr>
<td>Kadazan-Dusun</td>
<td>Control</td>
<td>6 (0.200)</td>
<td>18 (0.600)</td>
<td>6 (0.200)</td>
</tr>
<tr>
<td>Bajau</td>
<td>Case subject</td>
<td>13 (0.245)</td>
<td>26 (0.491)</td>
<td>14 (0.264)</td>
</tr>
<tr>
<td>Bajau</td>
<td>Control</td>
<td>8 (0.286)</td>
<td>14 (0.500)</td>
<td>6 (0.201)</td>
</tr>
<tr>
<td>Total</td>
<td>Case subject</td>
<td>56 (0.301)</td>
<td>99 (0.532)</td>
<td>31 (0.167)</td>
</tr>
<tr>
<td>Total</td>
<td>Control</td>
<td>36 (0.234)</td>
<td>84 (0.545)</td>
<td>34 (0.221)</td>
</tr>
</tbody>
</table>

Bold values represent significant $p$ values.
abuse (Itoh et al., 2005). In our study, although this association was not significant overall, the data for the Chinese subgroup suggested that the BDNF Val66Met polymorphism contributes to methamphetamine abuse vulnerability and methamphetamine dependence in this ethnicity. The risk for methamphetamine dependence in the Chinese subgroup with the 66Val 196G allele was 2.6, whereas with the 66Val homozygous 196G, it was 4.6, suggesting that this allele may contribute to methamphetamine dependence in the male Chinese population.

Although the results of this study showed that the 66Val allele for the Chinese subpopulation is a risk factor for methamphetamine dependence, the age of onset for methamphetamine abuse from either the overall data or the Chinese dependent subjects within the three genotypes was not significant, inconsistent with the results of Cheng et al. (2005). They reported a significant difference in the age of onset for methamphetamine abuse across the three genotype groups (p = 0.048), perhaps because of involvement of independent genes with a different penetrance level of different susceptibility loci in the pathogenesis of substance dependence.

In this study, the 66Met allele was less common in the Chinese methamphetamine-dependent groups than it was in the control group. However, the 66Val/66Val genotype is more common in methamphetamine-dependent groups, and this finding is also compatible with the observation from previous studies which was in Han Chinese population that the Met allele is the dominant allele of the BDNF Val66Met polymorphism (Cheng et al., 2005; Chen et al., 2004). Furthermore, the odds ratio of being methamphetamine dependent between 66Val/66Val and 66Val/66Met plus the 66Met/66Met genotype (OR=4.2) in this study also revealed that the Met allele is the dominant allele of the BDNF Val66Met polymorphism. This finding may indicate that the Chinese population in Malaysia and the Han Chinese have a common origin. The 66Val allelic frequency in our study for the Malaysian Chinese subgroup was 46.4%, comparable with previous findings that showed that the frequency of this allele in normal African-American, European-American, and Chinese people was 13.6%, 33.6%, and 46.7%, respectively. This result suggests that this allele is dominant in the Chinese and European-American population (Liu et al., 2005; Cheng et al., 2005).

Our overall data and stratified analyses by ethnicity showed that the BDNF Val66Met polymorphism was not associated with methamphetamine psychosis among the methamphetamine abusers, suggesting that this polymorphism does not cause susceptibility to psychosis in male Malaysian methamphetamine-dependent subjects. This finding is in line with the study of Itoh et al. (2005) that showed that the BDNF gene was not associated with methamphetamine psychosis in a Japanese population. Results of this study however, showed a significant difference in allele frequency between those who developed psychosis and those who do not in the Chinese methamphetamine-dependent subjects.

In contrast with the methamphetamine dependence, the risk for methamphetamine psychosis with the 66Val 196G allele was 0.2, while with 66Met 196A allele was 4.2 in Chinese methamphetamine-dependent subjects, whereas the methamphetamine dependence with the 66Val 196G allele was 2.6, suggesting that this 66Val allele is more likely to contribute to methamphetamine dependence in the male Chinese population but not for the methamphetamine psychosis. Moreover, 66Met allele may contribute to methamphetamine psychosis but not to methamphetamine dependence. This may be due to variation in definitions of psychosis that is being used in the present study compared to the ones that are being used in other studies, and it may, in part, be due to the small sample size of the methamphetamine psychosis in the present study.

Hall et al. (2003) in an animal study showed that heterozygous BDNF-knockout mice displayed cocaine-conditioned place preferences and reduced locomotion during habituation after cocaine injections. He reported that, compared with 66Met carriers, 66Val/66Val carriers may have higher levels of central BDNF, which increases the euphoric effect following methamphetamine administration and renders them more vulnerable to methamphetamine abuse. Our findings were similar to those of Hall et al. (2003) in that more case subjects than control subjects had the 66 Val variant.

There may be several explanations for the contrasting finding between the present study and that of Cheng et al. (2005). It is possible that our finding is a false positive which is contributed by the impact of stratification on our results.

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Participant</th>
<th>Genotype</th>
<th>Allele frequency</th>
<th>OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G/G</td>
<td>G/A</td>
<td>A/A</td>
</tr>
<tr>
<td>Malay</td>
<td>Psychosis</td>
<td>10 (0.357)</td>
<td>15 (0.536)</td>
<td>3 (0.107)</td>
</tr>
<tr>
<td></td>
<td>No psychosis</td>
<td>10 (0.323)</td>
<td>18 (0.581)</td>
<td>3 (0.097)</td>
</tr>
<tr>
<td>Chinese</td>
<td>Psychosis</td>
<td>2 (0.182)</td>
<td>7 (0.636)</td>
<td>2 (0.182)</td>
</tr>
<tr>
<td></td>
<td>No psychosis</td>
<td>8 (0.615)</td>
<td>5 (0.385)</td>
<td>0 (0.000)</td>
</tr>
<tr>
<td>Kadazan-Dusun</td>
<td>Psychosis</td>
<td>3 (0.214)</td>
<td>8 (0.572)</td>
<td>3 (0.214)</td>
</tr>
<tr>
<td></td>
<td>No psychosis</td>
<td>10 (0.278)</td>
<td>20 (0.555)</td>
<td>6 (0.167)</td>
</tr>
<tr>
<td>Bajau</td>
<td>Psychosis</td>
<td>4 (0.267)</td>
<td>8 (0.533)</td>
<td>3 (0.200)</td>
</tr>
<tr>
<td>Total</td>
<td>No psychosis</td>
<td>9 (0.237)</td>
<td>18 (0.474)</td>
<td>11 (0.289)</td>
</tr>
</tbody>
</table>

**Table 2 – Genotype and allelic frequencies of the BDNF Val66Met polymorphism in methamphetamine-dependent subjects with and without psychosis.**

p values.

* The p value was calculated by using Fisher’s exact test.
However, this is not very likely because we do have a relatively homogenous group, especially in gender and the type of drug used, all our subjects being males (Table 3) who were on methamphetamine only.

In conclusion, our results failed to show any association between BDNF Val66Met polymorphism with occurrence of methamphetamine dependence, and with risk of psychosis in the total Malaysian population studied. However, a significant difference in the allelic and genotype frequencies was found in the Malaysian Chinese population for both methamphetamine dependence and psychosis. Our findings suggest that the 66Val/66Val genotype of the BDNF Val66Met polymorphism is a risk factor for methamphetamine dependence. Besides that, the BDNF 66Met allele may contribute to a vulnerability to methamphetamine psychosis in the Chinese population. Further study with a larger sample size may provide more evidence to confirm the genetic influence of BDNF in methamphetamine dependence.

4. Experimental procedures

4.1. Subject recruitment and sample collection

Samples from the case-control study were obtained from the psychiatric unit at the University Malaya Medical Centre (UMMC) and from a drug rehabilitation centre in Papar, Sabah that specializes in methamphetamine-dependent patients. The subjects included all patients who fulfilled the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV; American Psychiatric Association, 1994) criteria for amphetamine and methamphetamine dependence. Methamphetamine dependence was confirmed by a positive urine test for methamphetamine during recruitment and the qualified psychiatrists from the two centres confirmed the presence or absence of psychosis. The subjects were considered to have psychosis if they have persecutory delusions and delusions of reference or present with auditory, visual hallucinations or tactile hallucinations. Subjects with mixed or unclear ethnicity and those with a history of psychiatric illness and other substance dependence were excluded.

The controls were obtained from healthy volunteers in the University of Malaya Medical Centre in Kuala Lumpur in West Malaysia and from the Luyang Health Clinic, Sabah in East Malaysia. They were medically healthy with no history of chronic medical or surgical illness, had no previous history of psychiatric illness, and did not fulfil the DSM-IV criteria for amphetamine and methamphetamine dependence. The sample size was calculated using an online programme, namely Power of Association With Errors (PAWE) (Gordon et al., 2002; Gordon and Nothnagel, 2003). A total of 186 subjects (n=186) and 154 controls (n=154) comprising Malay, Chinese, Kadazan-Dusun, and Bajau ethnicities were recruited and consented to the study. Approval for the study was obtained from the UMMC Ethics Committee.

4.2. DNA preparation and analysis

Three millilitres of blood was collected from each participant by a standard method in an EDTA tube. DNA was extracted from leucocytes by using the QIAmp Blood Kit (Qiagen, Germany). In cases in which the participant was reluctant to provide blood samples, buccal swab tissues were obtained by the QIAmp Mini Kit (Qiagen, Germany). Genotyping of the Val66Met genetic polymorphism of the BDNF gene (G196A; rs6265) was performed by using polymerase chain reaction (PCR)-based methods with forward and reverse primers (forward, 5′-ACTCTGGAGAGCT-GAAT-3′; reverse, 5′-ATACCTGTCACACCGCTC-3′, respectively). The PCR reaction was performed under the following conditions: 95 °C for 5 min; then 35 denaturing cycles of 30 s each at 95 °C, 30 s of annealing at the appropriate temperature, and 30 s each at 72 °C for extension, and final elongation at 72 °C for 10 min. PCR was carried out by using PCR Master Mix (Fermentas International Inc, Canada). Following that, the restriction fragment length polymorphism (RFLP) method was conducted with restriction enzyme NlaIII (New England Biolabs, USA). The PCR product was digested by NlaIII and the mixture was incubated at 37 °C in a dry-block heater overnight. The temperature was then increased to 65 °C for 20 min to terminate the activity of the enzyme. After the DNA was digested by restriction enzyme, products were loaded on 2.5% (w/v) agarose gel stained with GelRed (Biotium, USA) and then electrophoresed. The gel was viewed under UV light to observe restriction patterns. The size and distribution of the band was used to determine the genotype of the DNA sample. The PCR products were chosen randomly for validation by DNA sequencing.

Table 3 - Demographic and clinical characteristics of the subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Malay (n=110)</th>
<th>Chinese (n=89)</th>
<th>Kadazan-Dusun (n=80)</th>
<th>Bajau (n=81)</th>
<th>Total (n=340)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>59 (100)</td>
<td>51 (100)</td>
<td>24 (100)</td>
<td>45 (100)</td>
<td>50 (100)</td>
</tr>
<tr>
<td>Male</td>
<td>31 (7.8)</td>
<td>36 (10.6)</td>
<td>40 (9.6)</td>
<td>30 (9.4)</td>
<td>26 (6.9)</td>
</tr>
<tr>
<td>Onset age of meth dependence (years), mean (SD)</td>
<td>25 (9.1)</td>
<td>31 (10.1)</td>
<td>22 (7.5)</td>
<td>21 (7.1)</td>
<td>15 (28.3)</td>
</tr>
<tr>
<td>Meth dependence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With psychosis, N (%)</td>
<td>28 (47.5)</td>
<td>11 (45.8)</td>
<td>14 (28)</td>
<td>15 (28.3)</td>
<td>15 (28.3)</td>
</tr>
<tr>
<td>Without psychosis, N (%)</td>
<td>31 (52.5)</td>
<td>13 (54.2)</td>
<td>36 (72)</td>
<td>38 (71.7)</td>
<td>68 (36.6)</td>
</tr>
</tbody>
</table>
4.3 Statistical analysis

Inter-group statistical analyses were performed by using the chi-square test and the Fisher’s exact test, where necessary, to compare each ethnic group’s cases with ethnically matched healthy controls for the frequencies of the BDNF 196G/G genotype, the 196G/A heterozygote, and the 196A/A genotype. The Fisher’s exact test was performed when sample sizes were too small. For all analyses, p values of less than 0.05 were considered statistically significant.

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