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BRIEF REPORT

Positive association of AKT1 haplotype to Japanese methamphetamine use disorder

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Abstract

Recent evidence suggests that the AKT1-GSK3β signalling cascade partially mediates dopamine-dependent behaviours. In relation to the pathophysiology of schizophrenia or methamphetamine (Meth) use disorder, AKT1 is a good candidate gene for such conditions. For schizophrenia, positive associations of SNPs and AKT1 haplotypes were reported in US and Japanese samples. To evaluate the association between AKT1 and Meth-use disorder, we conducted a case-control study of Japanese samples (182 patients and 437 controls). A positive association between a SNP and haplotypes was found, and the 'signal' SNP was the same SNP found to be associated with US schizophrenia, but not with Japanese schizophrenia. Our results indicate that AKT1 may play a possible role in the development of Meth-use disorder. Further investigation of these associations, together with evidence from previous animal studies, may open the way to elucidation of the pathophysiology of this condition.

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Key words: Dopamine-dependent behaviours, linkage disequilibrium, substance-related disorders.

Introduction

The pathophysiology of methamphetamine (Meth) use disorder has not been well established, however, one of the most likely mechanisms is abnormality of the dopamine (DA) neurotransmission system. The pharmacological profile of Meth shows that the target site of Meth is the DA transporter (DAT). Also the mesolimbic DA system has an important function in reinforcement and reward mechanisms (Spanagel and Weiss, 1999).

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Family and twin studies suggested that the genetic contribution is important in that it may predispose certain people to this disorder (Tsuang et al., 1996, 1998). Recent studies have suggested that V-akt murine thymoma viral oncogene homologue 1 (AKT1) is a good candidate for a Meth-use disorder susceptibility gene, for the following reasons. (1) An animal study of DAT knock-out (KO) mice and wild-type mice, treated with lithium salts and amphetamine, showed that the AKT1–glycogen synthase kinase 3β (GSK3β) signalling cascade partially mediated DA-dependent behaviours (Beaulieu et al., 2004). (2) AKT1 KO mice treated with amphetamine showed a reduction in prepulse inhibition (PPI) (Emamian et al., 2004). PPI disturbances are known to be present in schizophrenia, which might also be related to

abnormalities in the DA system. AKT1 haplotypes were shown to have a significant association with schizophrenia in a transmission disequilibrium test (TDT) study (Emamian et al., 2004) and in a previous Japanese case-control replication study by the authors (Ikeda et al., 2004), although no association was found in another Japanese replication study (Ohtsuki et al., 2004).

Here we conducted a case-control study of Japanese Meth-use disorder samples using the single nucleotide polymorphisms (SNPs) of our previous study to evaluate the association of AKT1 with Meth-use disorder.

Methods

A total of 182 patients with Japanese Meth-use disorder [146 male, 36 female; mean age $\pm$ standard deviation (S.D.), 36.7 ± 12.0 yr] and 437 controls (209 male, 228 female; 34.3 ± 13.6 yr) were analysed. The number of patients with Meth-use disorder comprised of 168 Meth-dependent subjects, and 14 Meth-abuse subjects. Among the subjects with Meth-use disorder, 153 subjects (127 males, 26 females) have a comorbid diagnosis of Meth-induced psychosis, three of anorexia nervosa, one of obsessive–compulsive disorder, and one of major depressive disorder. And 120 subjects with Meth-use disorder have abuse or dependence on drugs other than Meth. Subjects with Meth-use disorder were excluded if they had a comorbid diagnosis of any psychotic disorder other than Meth-induced psychosis. They were diagnosed according to DSM-IV criteria by the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and review of the medical records. All healthy controls were also psychiatrically screened based on unstructured interviews. More details about the characterization of these subjects have been published elsewhere (Suzuki et al., 2003; Ujike et al., 2003). After description of the study, written informed consent was obtained from each subject. This study was approved by the Ethics Committee at each participating institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA).

For SNP genotyping, polymerase chain reaction (PCR) amplification, restriction fragment length polymorphism (RFLP) assays were developed; *Bsa*I for SNP1 (rs3803300), *Xcm*I for SNP2 (rs1130214), *Hae*III for SNP3 (rs3730358), *Hpy*CH4IV for SNP4 (rs2498799), *Pst*I for SNP5 (rs2494732), and *Bsi*HKAI for SNPA (rs2498804). A detailed description may be found in a previous report (Ikeda et al., 2004) and information about primer sequences and PCR–RFLP conditions are available on request.

Hardy–Weinberg equilibrium (HWE) was evaluated by conventional χ^2 test (SPSS 10.0J, SPSS Japan Inc., Tokyo, Japan). For marker-trait association analyses, we constructed multi-SNP haplotype systems (Emamian et al., 2004) to evaluate the association through permutation p values in sliding window fashion and global p values respectively. In total sample association analysis (not in explorative association analysis), we emphasize the permutation p values over the respective global p values because the permutation procedure gives a significance corrected for the multiple haplotypes and markers tested. Furthermore, we corrected these permutation p values by Bonferroni correction to obtain more robust results. A more detailed description is given in our previous paper (Ikeda et al., 2004).

We also include an explorative analysis for gender effects, because of the following reasons. (1) Aetiological study suggests that the genetic contribution of substance-related disorder is differentially heritable by gender (Jang et al., 1997). (2) Our samples were unmatched gender ratios of Meth-use disorder (36 female, 146 male).

Results

Genotype frequencies of all SNPs were in HWE. Positive permutation p values of 4- and 5-marker sliding window fashion ($p = 0.0083$ and 0.023 respectively) and global p value of 6-marker combinations ($p = 0.017$) were obtained. One of the 4-marker sliding window fashion p values remained significant ($p = 0.0498$) even after Bonferroni correction was performed six times (once for single marker permutation and five times for haplotype combinations). In the single marker association analysis (i.e. a conventional allele-wise association analysis), only SNP3 was associated with Meth-use disorder ($p = 0.019$) (Table 1).

Individual haplotypic analyses from the positive global 4-marker p values are shown in Table 2. The haplotype with the most significant association was more frequent in controls than in cases (SNP1-2-3-4, G-G-C-G, $p = 0.0032$).

Explorative analysis of gender effects is shown in Table 3. In female samples, eight of 21 global p values showed significance. In these significant p values, SNP3, which was associated with total Meth-use disorder, showed strong association ($p = 0.0011$). On the other hand, the positive global p values in male samples tended to be similar to those in total samples (positive global p values: SNP1-2-3-4 = 0.036, SNP1-2-3-4-5-A = 0.042), however, SNP3 was not associated with male Meth-use disorder ($p = 0.11$).

Table 1. Association analyses of the AKT1 gene

							Genotypic distribution					
	Multi-SNP haplotype systems						M/M		M/m		m/m	
SNP ID	1 SNP	2 SNP	3 SNP	4 SNP	5 SNP	6 SNP	Meth	Control	Meth	Control	Meth	Control
SNP1 (rs3803300G > A)	0.15						63	124	91	234	28	79
		0.22										
SNP2 (rs1130214G > T)	0.97		0.096				128	315	51	108	3	14
		0.27		**0.0023**								
SNP3 (rs3730358C > T)	**0.019**		0.43		**0.0082**		136	364	43	68	3	5
		0.12		0.23		**0.017** (0.10)						
SNP4 (rs2498799G > A)	0.81		0.11		0.16		40	121	98	211	44	105
		0.53		0.063								
SNP5 (rs2494732A > G)	0.59		0.19				86	212	79	192	17	33
		0.16										
SNPA (rs2498804T > G)	0.20						63	142	92	206	27	89
Permutation *p* value	*0.097*	*0.40*	*0.28*	***0.0083*** (***0.0498***)	***0.023*** (*0.14*)							

p values were calculated by log likelihood ratio test (SNP1, allele-wise association; SNP2-6, global haplotypic association).
M, major allele; m, minor allele; Meth, methamphetamine-use disorder.
Bold values represent significant *p* values.
Values within parentheses represent *p* values after Bonferroni correction.

Table 2. Haplotype frequencies from positive permutation analysis

Combination of SNPs	Marker haplotype	Frequency		*p* values
		Meth	Control	
SNP1-2-3-4	A-G-C-G	0.28	0.20	0.023
	A-G-T-A	0.074	0.048	0.049
	G-G-C-G	0.12	0.21	0.0032

Meth, Methamphetamine-use disorder.

Discussion

A positive association between a SNP and AKT1 haplotypes was found in our Japanese Meth-use disorder samples. In assessing the components of these associations, we considered SNP3 to be a main component associated with Meth-use disorder, because the single marker association of SNP3 was significant in total samples ($p = 0.019$). Interestingly, this SNP was associated with US schizophrenia in an original TDT analysis (Emamian et al., 2004). On the contrary, we found no association SNP3 to Japanese schizophrenia in a previous study (SNP5 was associated with Japanese schizophrenia) (Ikeda et al., 2004). This difference in predisposing SNPs between Japanese Meth-use disorder and Japanese schizophrenia might be explained by their respective linkage disequilibrium (LD) patterns. We have shown that the LD pattern in schizophrenia was slightly different from that in controls, while the pattern in Meth-use disorder tended to be similar to that in control samples (data not shown). These findings indicate that different predisposing polymorphisms may exist independently in schizophrenia and Meth-use disorder, and may be located in LD with SNP5 or SNP3 respectively.

The result of explorative analysis might support the 'gender effects' of Meth-use disorder, reported in a previous genetic association study of Meth-use disorder (Lin et al., 2003). Especially, female samples of

Table 3. Explorative analysis of gender effects

		Multi-SNP haplotype systems					
Gender	SNP ID	1 SNP	2 SNP	3 SNP	4 SNP	5 SNP	6 SNP
Female ($n=36$)	SNP1	0.55					
			0.41				
	SNP2	0.084		**0.017**			
			0.012		**0.012**		
	SNP3	**0.0011**		**0.049**		**0.028**	
			0.028		0.084		**0.023**
	SNP4	0.50		0.076		0.057	
			0.67		0.16		
	SNP5	0.34		0.79			
			0.57				
	SNPA	0.73					
Male ($n=146$)	SNP1	0.23					
			0.13				
	SNP2	0.28		0.18			
			0.51		**0.036**		
	SNP3	0.11		0.47		0.053	
			0.26		0.41		**0.042**
	SNP4	0.58		0.19		0.22	
			0.55		0.078		
	SNP5	0.40		0.12			
			0.30				
	SNPA	0.50					

Bold values represent significant p values.

Meth-use disorder were strongly associated with a SNP and haplotypes of AKT1, while male samples were weakly associated. However, because the sample size of female subjects was small ($n=36$), a type I error might occur in this explorative analysis. Even assuming that there are no 'gender effects' of AKT1, the fact remains that AKT1 is associated with Meth-use disorder. In this case, these association analyses of total and divided samples indicate the following interpretations. (1) SNP3 might not be an 'actual' predisposing SNP by itself, nor be in perfect LD with 'actual' predisposing polymorphisms, because male samples with Meth-use disorder were not associated with SNP3 (only total or female samples were associated with it). (2) At least some haplotypes of AKT1 may play a possible role in the development of Meth-use disorder, because two haplotypes of AKT1, including the combination of SNP1-2-3-4 and SNP 1-2-3-4-5-A, are associated with Meth-use disorder both in divided samples and total samples.

Our results had several limitations in terms of interpreting positive associations. (1) The positive associations we detected might be due to type I error, possibly because of population stratification, an unmatched-gender sample and small sample size, described above. Larger sample size and genomic control would be required. (2) Type I error might also have occurred because of multiple testing. We corrected p values by applying a permutation procedure and Bonferroni correction in total samples. However, in individual haplotypic analysis or explorative analysis of gender effects, we did not apply each correction. At this time, an optimal method for resolving this problem (correction among global and individual haplotypic analysis, or explorative-subgroup analysis) has not yet been established. Greater knowledge of genetic models and more useful methods would be required to re-analyse these results. (3) The other confounding factors such as ascertainment bias can account for the apparent association in this study. For example, because subjects with Meth-induced psychosis consisted of the majority of our samples, this condition would be over-represented in our samples of Meth-use disorder. Further explanation is given in Nishiyama et al. (2005).

Our results indicate that AKT1 may play a role in the development of Meth-use disorder. Our findings also support the hypothesis that abnormalities in AKT1 might contribute to the pathophysiology of DA-dependent behaviour such as Meth-use disorder and schizophrenia. Further studies including mutation search to detect 'actual' predisposing polymorphisms and functional analysis of AKT1 (or its cascade) may open the way to elucidation of the pathophysiology of this condition.

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Statement of Interest

None.

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