

Forum Minireview

New Perspectives in the Studies on Endocannabinoid and Cannabis: Cannabinoid Receptors and Schizophrenia

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Abstract. Cannabis consumption may induce psychotic states in normal individuals, worsen psychotic symptoms of schizophrenic patients, and may facilitate precipitation of schizophrenia in vulnerable individuals. Recent studies provide additional biological and genetic evidence for the cannabinoid hypothesis of schizophrenia. Examinations using [³H]CP-55940 or [³H]SR141716A revealed that the density of CB₁ receptors, a central type of cannabinoid receptor, is increased in subregions of the prefrontal cortex in schizophrenia. Anandamide, an endogenous cannabinoid, is also increased in the CSF in schizophrenia. A genetic study revealed that the CNR1 gene, which encodes CB₁ receptors, is associated with schizophrenia, especially the hebephrenic type. Individuals with a 9-repeat allele of an AAT-repeat polymorphism of the gene may have a 2.3-fold higher susceptibility to schizophrenia. Recent findings consistently indicate that hyperactivity of the central cannabinoid system is involved in the pathogenesis of schizophrenia or the neural mechanisms of negative symptoms.

Keywords: cannabinoid, CB₁ receptor, anandamide, CNR1 gene, schizophrenia

Introduction

Schizophrenia is the second most common mental illness after depression. It typically begins in late adolescence or early adulthood with characteristic psychiatric symptoms, for example, delusions and/or hallucinations, loose association, blunted or inappropriate affect, and distortions of perception. The disorder is a chronic and severe mental illness with a lifetime prevalence of about 1% worldwide. Behavior may be seriously impaired, leading to adverse social consequences. Recent development of typical and atypical neuroleptics has produced great improvement in the clinical symptoms of patients, but it is still inadequate, and the overall prognosis for schizophrenia is still far from satisfactory. Such unsuccessful treatment must result, at least partly, from insufficient understanding of the pathogenesis of schizophrenia. To date, various hypotheses for the etiology of schizophrenia have been proposed, for example, the classical dopamine hypothesis, the NMDA-receptor hypothesis, and the current

neurodevelopmental hypothesis. Among them, the “cannabinoid hypothesis”, which was originally based on clinical findings in marijuana abusers, has been developed as one of the pharmacological etiologies for schizophrenia.

Cannabis consumption and schizophrenia

There have been a number of case reports indicating that consumption of a relatively large amount of cannabis could precipitate a psychotic state called “cannabinoid psychosis”, with hallucinations, delusions, and emotional liability, resembling schizophrenia (1–4). An Indian study showed that the most potent cannabis preparations produced psychotic symptoms after the shortest period of consumption in cannabis abusers (5). Administration of Δ^9 -tetrahydrocannabinol (Δ^9 -THC), a major ingredient of cannabis, to normal volunteers induced cognitive impairment in three-dimensions closely resembling that of schizophrenia patients (6). These clinical studies indicate that cannabis may have psychotomimetic effects in previously non-psychotic subjects.

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In schizophrenic patients, abuse of cannabis has worsened positive symptoms of schizophrenia (7, 8), even under a regular regimen of antipsychotics (9). Cannabinoid consumption by schizophrenics results in a poor outcome and liability to relapse (10, 11). A prospective study over one year showed that psychotic patients who used cannabis relapsed to a psychotic state sooner and more frequently than patients who did not use cannabis. A dose-response relationship in relapse was also observed (12).

It is possible that cannabis use does not merely precipitate cannabis psychosis or exacerbate schizophrenic symptoms. It may also precipitate endogenous psychosis of schizophrenia in individuals who are vulnerable to the psychosis. Several clinical studies showed high rates of cannabis consumption in schizophrenic patients (13, 14). Schizophrenic patients with a positive urine test for cannabis at admission had a family history of psychosis more frequently than patients with a negative urine test (15). The Edinburgh high-risk study group showed that frequent cannabis use was associated with a six-fold increase in the risk of psychosis in high risk individuals who have a strong family history of psychosis compared with patients without a family history (16). The strongest epidemiological evidence was provided by a Swedish cohort study. Andreasson et al. found that cannabis use before the age 18 years was associated with increased risk of schizophrenia 15 years later (Fig. 1). The relative risk of precipitation of schizophrenia was 2.4 times higher than that of someone who did not use cannabis before the age of 18 years (17). A dose-response relationship was also demonstrated because heavy consumption (more than 50 times by age 18) was associated with a sixfold increase in the incidence of schizophrenia. These findings indicate that enhancement of the cannabinoid system by cannabis consumption produces *de novo* psychosis in normal individuals, worsens psychotic

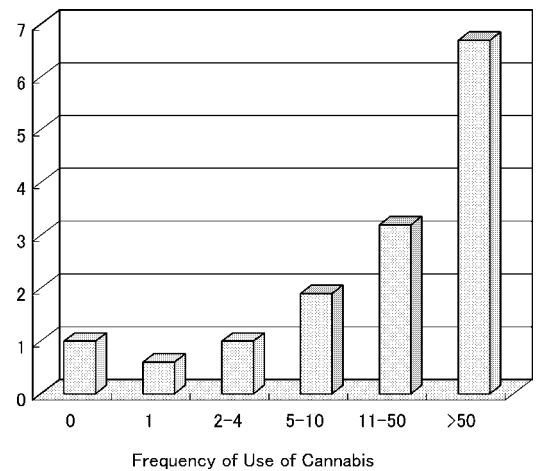


Fig. 1. Epidemiologic study of morbidity risk of cannabis use before 18 year for development of schizophrenia. Bar graph is based on the data of Andreasson et al. (1987) (Ref. 17). The vertical axis indicates the odds ratio of schizophrenia.

symptoms previously seen in schizophrenic patients, and may facilitate precipitation of endogenous psychosis of schizophrenia, especially in individuals who have vulnerability to psychosis.

Postmortem study of brain cannabinoid receptors

In 1988, the existence of specific binding sites for cannabinoids in the brain were discovered and designated as the central cannabinoid receptors or CB₁ receptors, which are coupled to G protein (18) (Table 1). A second type of cannabinoid receptor, designated peripheral cannabinoid receptors or CB₂ receptors, was subsequently found to exist in the spleen and immune system. Therefore, the psychotomimetic effects of cannabis and marijuana must be mediated via CB₁ receptors. Two independent groups have measured CB₁-receptor densities in schizophrenic brains postmortem.

Table 1. Subtypes of cannabinoid receptors

	CB ₁ receptor	CB ₂ receptor
Amino acids	472 AA	360 AA
Locus	6q14–q15	1p36.11
Gene name	CNR1	CNR2
Endogenous ligand	2-Arachidonoylglycerol, Anandamide	N-Palmitylethanolamine
Distribution		
CNS	Substantia nigra, Putamen, Hippocampus, Cerebellum, Cerebral cortex	none
Peripheral	Testis, Uterus, Lung	Spleen, Tonsile, Lymph Node
Physiology	Spatial cognition, Mood elevation, Short-term memory, Substance dependence	Inflammation, Immune function?

Dean et al. (19) used in situ radioligand binding and autoradiography to measure [3 H]CP-55940, a non-selective cannabinoid agonist, in the dorsolateral prefrontal cortex (Brodmann's area 9), caudate-putamen, and areas of the temporal lobe and found a significant increase in [3 H]CP-55940 binding in the dorsolateral prefrontal cortex in subjects with schizophrenia that was independent of recent cannabis ingestion. They also found an increase in the density of CB₁ receptors in the caudate-putamen that was independent of diagnosis in subjects who had recently ingested cannabis. They speculated that changes in CB₁ receptors in the dorsolateral prefrontal cortex may be associated with the pathology of schizophrenia. Zavitsanou et al. (20) used [3 H]SR141716A as a radioligand for quantitative autoradiography of CB₁ receptors because SR141716A is a more potent and selective ligand for CB₁ receptors than CP-55940. They examined the anterior cingulate cortex, which plays an important role in normal cognition, particularly in relation to motivation and attention, and found a statistically significant 64% increase in [3 H]SR141716A-specific binding in the schizophrenia group as compared to the control group. These two independent postmortem studies showed an increase of CB₁ receptors in subregions of the prefrontal cortex, dorsolateral and anterior cingulate regions in schizophrenia. The major clinical symptoms observed in schizophrenia are classified into three categories: positive, negative, and disorganized symptoms. Negative symptoms consist of blunting of affect, poverty of speech and thought, apathy, anhedonia, reduced social drive, and loss of motivation. Such negative symptoms, including cognitive impairment, may result from dysfunction of the prefrontal cortex. Because cannabinoids disarrange cognition, attention, and motivation, it is possible that the increased numbers of CB₁ receptors in the prefrontal cortex may be involved in the pathology of schizophrenia particularly in relation to negative symptoms.

Endocannabinoids in cerebrospinal fluid (CSF)

Following the discovery of cannabinoid receptors in the brain, endogenous ligands for cannabinoid receptors were found in mammalian tissues. These are arachidonic acid derivatives. To date, two molecules have been identified as endocannabinoids acting on CB₁ receptors: anandamide (*N*-arachidonyl-ethanolamine) (21) and 2-AG (2-arachidonylglycerol) (22). They are able to reproduce the most typical behavioral effects of Δ^9 -THC, such as inhibition of locomotor activity, analgesia on a hot plate, and hypothermia, in rodents. Therefore, they act as agonists in vivo for the cannabinoid systems.

Leweke et al. (23) examined CSF concentrations of endocannabinoids in schizophrenia. They found that CSF levels of anandamide and palmitylethanolamide, another endocannabinoid acting mainly on CB₂ receptors, were increased by twofold in schizophrenic patients compared to non-schizophrenic controls, and 2-AG in the CSF was below detection in both groups. The increase of the two endocannabinoids in the CSF of schizophrenics was not affected by medication. Because increases in the CSF levels of anandamide and palmitylethanolamide should reflect increases in the brain, hyperactivity of the endocannabinoid system in the central nervous system may be involved in the pathogenesis of schizophrenia.

Genetic studies

CB₁ receptors are encoded by the CNR1 gene (MIM114610), which was cloned by Matsuda et al. in 1992 (24). CB₁ is located at 6q14–q15, a site including a schizophrenia susceptibility locus, 6q13–q26, which was designated as the Schizophrenia 5 locus (SCZ5, OMIM 603175), as revealed by Cao et al. (25) using two independent series of pedigrees. Two polymorphisms, an AAT-repeat microsatellite in the 3' flanking region and a 1359 G/A polymorphism at codon 453, a silent mutation, in the coding exon of the CNR1 gene, were reported previously. Additional screening of the promoter and entire coding region of the CNR1 gene by direct sequencing revealed two novel polymorphisms, 843G/C and L352S (26); however, their heterozygosities were as low as 1%, and the estimated genetic significance may be small. Therefore, the association with schizophrenia was studied using the AAT repeat and 1359 G/A polymorphisms of the CNR1 gene (27). Although the genotypic and allelic distribution of the 1359 G/A polymorphism was not different in schizophrenic and control subjects in a Japanese population, which is consistent with a Caucasian study (28), allelic distributions of the AAT-repeat polymorphism differed significantly between them (Fig. 2). Schizophrenia is usually divided into three subtypes by clinical features: paranoid, hebephrenic, and catatonic types. The hebephrenic subtype of schizophrenia, but not others, showed a strong association with the CNR1 gene ($P = 0.0028$). The 9-repeat allele of the AAT-repeat polymorphism was a genetic risk factor for susceptibility to hebephrenic schizophrenia (odds ratio = 2.3), and the 17-repeat allele of it was a strong negative risk factor, thus protecting against susceptibility to hebephrenic schizophrenia (odds ratio = 0.21). Namely, an individual with a 9- or 17-repeat allele should have a risk of hebephrenic schizophrenia increased by about twice

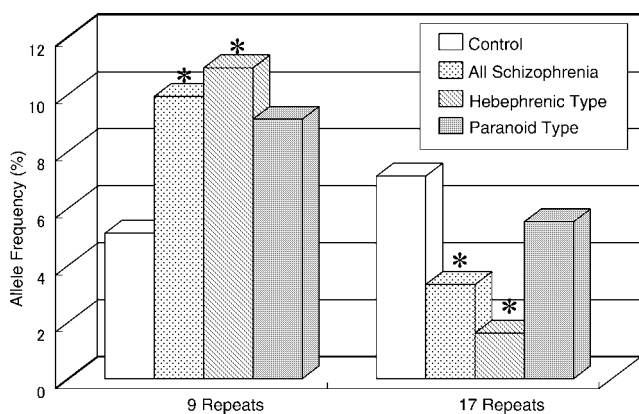


Fig. 2. Association of AAT-repeat polymorphism of the CNR1 gene with schizophrenia. * $P < 0.05$. Data from Ref. 27.

and decreased by one fifth, respectively, compared to an individual without either allele. This finding was not supported by a Chinese study (29), in which schizophrenia was examined only in total rather than by subtypes. Although further investigation in different populations is needed to confirm the significance of the CNR1 gene as a genetic risk factor for hebephrenic schizophrenia, the relationship between hebephrenic schizophrenia and the CNR1 gene is very intriguing. Clinical studies have reported that long-term cannabis use causes a combination of symptoms including cognitive impairment, lack of motivation, and impaired attention, the so-called “amotivational syndrome,”

which resembles the core negative symptoms of schizophrenia (30). Hebephrenic schizophrenia is characterized by disorganized symptoms and also progressive deterioration of negative symptoms such as blunted affect and abulia. We have also consistently found that the CNR1 gene is not associated with methamphetamine psychosis, which shows resemblance to the paranoid type but not the hebephrenic type of schizophrenia, because it has few negative symptoms (unpublished data). The genetic evidence indicates that enhanced endocannabinoid activity may be involved in the negative symptoms of schizophrenia.

Fatty acid amide hydrolase (FAAH, MIM 602935) serves as a primary and rapid catabolic regulator of anandamide, 2-AG, and related fatty acid amide-signaling molecules in vivo (31). FAAH knock-out mice exhibit a 15-fold augmentation of endogenous brain levels of anandamide and an array of intense CB₁-receptor-dependent behavioral responses, including hypomotility, analgesia, catalepsy, and hypothermia (32). Recently, the Pro129Thr missense and functional mutation was identified in the FAAH gene. Sipe et al. (33) found that its homozygous form is strongly associated with both street-drug use and problem drug/alcohol use. However, we have found that there is no association between the Pro129Thr mutation of the FAAH gene and susceptibility to schizophrenia in a Japanese population (34). Possible involvement of the gene in negative symptoms of schizophrenia including cognitive function should be elucidated.

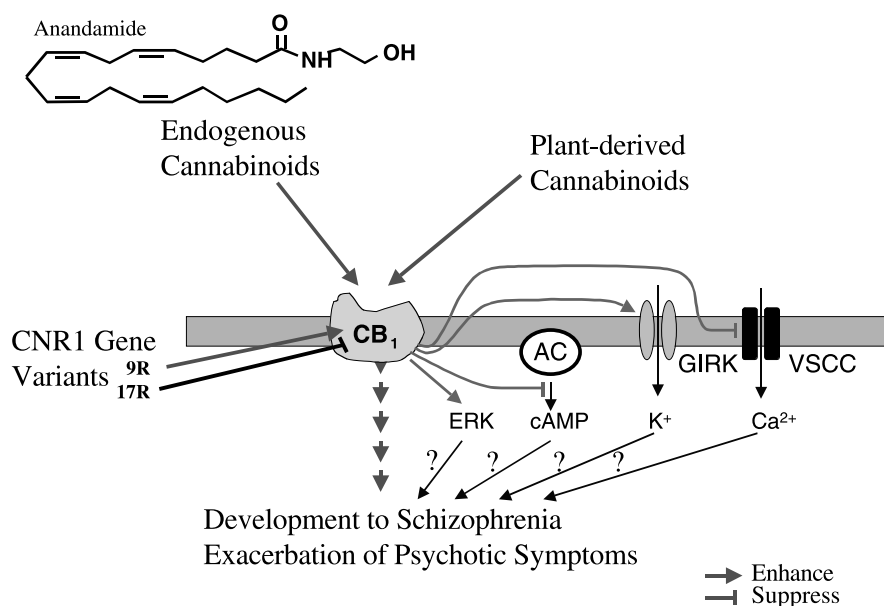


Fig. 3. Cannabinoid system and schizophrenia.

Conclusion

Several lines of biological and genetic evidence that support the cannabinoid hypothesis for schizophrenia are summarized in Fig. 3. Enhanced signaling of the cannabinoid system due to heavy consumption of exogenous cannabis, for example, marijuana, or increased endocannabinoid anandamide, which is mediated by CB₁ receptors in the brain, could precipitate schizophrenia. Increased CB₁ receptor density in the prefrontal cortex of schizophrenic patients should facilitate neural transmission of cannabinoids. In addition, genetic variants of the CNR1 gene, which encodes CB₁ receptors, could alter the efficiency of the receptor function or expression rate of the receptor molecules. All of the various findings observed in schizophrenia could converge into hyperactivity of the cannabinoid systems in the brain, which should play an important role in the pathogenesis of schizophrenia. Particularly, it is most significant clinically that the possible involvement of the cannabinoid system in the neural basis for the negative symptoms of schizophrenia. The recent development of atypical neuroleptics, for example, clozapine, olanzapine, and risperidone, is greatly beneficial to schizophrenic patients because they have been reported to improve not only positive but also negative symptoms with fewer adverse effects than conventional neuroleptics. However, the degree of improvement is not sufficient, especially in negative symptoms. More effective therapy for negative symptoms should be sought. The cannabinoid hypothesis of schizophrenia could be a key to development of an innovative therapy and comprehensive understanding of the etiology of schizophrenia.

References

1 Halikas JA, Goodwin DW, Guze SB. Marijuana use and psychiatric illness. *Arch Gen Psychiatry*. 1972;27:162–165.

2 Spencer DJ. Cannabis-induced psychosis. *Int J Addict*. 1971;6:323–326.

3 McGuire PK, Jones P, Harvey I, Bebbington P, Toone B, Lewis S, et al. Cannabis and acute psychosis. *Schizophr Res*. 1994;13:161–167.

4 Johns A. Psychiatric effects of cannabis. *Br J Psychiatry*. 2001;178:116–122.

5 Chopra GS, Smith JW. Psychotic reactions following cannabis use in East Indians. *Arch Gen Psychiatry*. 1974;30:24–27.

6 Emrich HM, Leweke FM, Schneider U. Towards a cannabinoid hypothesis of schizophrenia: cognitive impairments due to dysregulation of the endogenous cannabinoid system. *Pharmacol Biochem Behav*. 1997;56:803–807.

7 Negrete JC. Cannabis and schizophrenia. *Br J Addict*. 1989;84:349–351.

8 Turner WM, Tsuang MT. Impact of substance abuse on the

course and outcome of schizophrenia. *Schizophr Bull*. 1990;16:87–95.

9 Treffert DA. Marijuana use in schizophrenia: a clear hazard. *Am J Psychiatry*. 1978;135:1213–1215.

10 Breakey WR, Goodell H, Lorenz PC, McHugh PR. Hallucinogenic drugs as precipitants of schizophrenia. *Psychol Med*. 1974;4:255–261.

11 Martinez-Arevalo MJ, Calcedo-Ordonez A, Varo-Prieto JR. Cannabis consumption as a prognostic factor in schizophrenia. *Br J Psychiatry*. 1994;164:679–681.

12 Linszen DH, Dingemans PM, Lenior ME. Cannabis abuse and the course of recent-onset schizophrenic disorders. *Arch Gen Psychiatry*. 1994;51:273–279.

13 Cohen M, Klein DF. Drug abuse in a young psychiatric population. *Am J Orthopsychiatry*. 1970;40:448–455.

14 Barbee JG, Clark PD, Crapanzano MS, Heintz GC, Kehoe CE. Alcohol and substance abuse among schizophrenic patients presenting to an emergency psychiatric service. *J Nerv Ment Dis*. 1989;177:400–407.

15 McGuire PK, Jones P, Harvey I, Williams M, McGuffin P, Murray RM. Morbid risk of schizophrenia for relatives of patients with cannabis-associated psychosis. *Schizophr Res*. 1995;15:277–281.

16 Miller P, Lawrie SM, Hodges A, Clafferty R, Cosway R, Johnstone EC. Genetic liability, illicit drug use, life stress and psychotic symptoms: preliminary findings from the Edinburgh study of people at high risk for schizophrenia. *Soc Psychiatry Psychiatr Epidemiol*. 2001;36:338–342.

17 Andreasson S, Allebeck P, Engstrom A, Rydberg U. Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet*. 1987;2:1483–1486.

18 Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol*. 1988;34:605–613.

19 Dean B, Sundram S, Bradbury R, Scarr E, Copolov D. Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience*. 2001;103:9–15.

20 Zavitsanou K, Garrick T, Huang XF. Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004;28:355–360.

21 Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*. 1992;258:1946–1949.

22 Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol*. 1995;50:83–90.

23 Leweke FM, Giuffrida A, Wurster U, Emrich HM, Piomelli D. Elevated endogenous cannabinoids in schizophrenia. *Neuroreport*. 1999;10:1665–1669.

24 Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*. 1990;346:561–564.

25 Cao Q, Martinez M, Zhang J, Sanders AR, Badner JA, Cravchik A, et al. Suggestive evidence for a schizophrenia susceptibility locus on chromosome 6q and a confirmation in an independent

- series of pedigrees. *Genomics*. 1997;43:1–8.
- 26 Iwara N, Suzuki T, Kitajima T, Yamanouchi Y, Ikeda M, Inada T, et al. Mutation screening of the human cannabinoid receptor 1 (CNR1) in Japanese schizophrenia. *Am J Med Genet*. 2003;122B:113.
 - 27 Ujike H, Takaki M, Nakata K, Tanaka Y, Takeda T, Kodama M, et al. CNR1, central cannabinoid receptor gene, associated with susceptibility to hebephrenic schizophrenia. *Mol Psychiatry*. 2002;7:515–518.
 - 28 Leroy S, Griffon N, Bourdel MC, Olie JP, Poirier MF, Krebs MO. Schizophrenia and the cannabinoid receptor type 1 (CB1): association study using a single-base polymorphism in coding exon 1. *Am J Med Genet*. 2001;105:749–752.
 - 29 Tsai SJ, Wang YC, Hong CJ. Association study of a cannabinoid receptor gene (CNR1) polymorphism and schizophrenia. *Psychiatr Genet*. 2000;10:149–151.
 - 30 Kupfer DJ, Detre T, Koral J, Fajans P. A comment on the “amotivational syndrome” in marijuana smokers. *Am J Psychiatry*. 1973;130:1319–1322.
 - 31 Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature*. 1996;384:83–87.
 - 32 Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, et al. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci USA*. 2001;98:9371–9376.
 - 33 Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. *Proc Natl Acad Sci USA*. 2002;99:8394–8399.
 - 34 Morita Y, Ujike H, Tanaka Y, Uchida N, Nomura A, Ohtani K, et al. A nonsynonymous polymorphism in the human fatty acid amide hydrolase gene did not associate with either methamphetamine dependence or schizophrenia. *Neurosci Lett*. In press.