American Journal of Medical Genetics (Neuropsychiatric Genetics) 96:797–800 (2000)

Brief Research Communication

Association of 5HT2A Receptor Gene Polymorphism and Alcohol Abuse With Behavior Problems

Hai-Gwo Hwu[1]* and Chia-Hsiang Chen[2]

[1]Department of Psychiatry, College of Medicine, National Taiwan University, Taipei, Taiwan
[2]Department of Psychiatry, Tzu Chi College of Medicine, Hualien, Taiwan

This study investigated the association between T/C polymorphism, at position 102, of the 5-hydroxytryptamine 2A receptor gene and alcoholism with and without behavior problems. Eighty-five subjects (45 men, 40 women) with alcohol abuse, 75 subjects (51 men, 24 women) with alcohol dependence, and 70 normal control subjects (21 men, 49 women) participated in the study. The results show that the frequency of the homozygous T102 genotype was significantly lower in the group of male alcohol abuse with behavior problems than in the female group (χ^2 = 4.072, df = 1, $P < 0.05$) and the allele frequency of T102 was also lower in the male group than in the female group (χ^2 = 4.187, df = 1, $P < 0.05$). Of the male alcohol abuse subjects, the group with behavior problems was found to have lower frequencies of the T102 allele than the group without behavior problems (χ^2 = 4.328, df = 1, $P < 0.05$). In conclusion, this study demonstrates that alcoholism is heterogeneous and male alcohol abuse with behavioral problems was associated with T/C 102 polymorphism of the 5HT2A receptor gene. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 96:797–800, 2000.

KEY WORDS: alcoholism; heterogeneity; serotonin receptor gene; behavior problem

INTRODUCTION

Genetic factors are found to be important in the etiological complex of alcoholism [Cloninger et al., 1981; Sigvardsson et al., 1996]. Type 1 alcoholism, defined by Cloninger et al. [1981], was found to have both environmental and genetic risk factors, and Type 2 alcoholism, the severe form of alcoholism, was found to have a more emphasized genetic etiological factor [Sigvardsson et al., 1996]. Serotonin was found to play important roles in mediating drinking behavior through various mechanisms [Meltzer, 1990; Le Marquand et al., 1994; Virkkunen and Linnoila, 1997; Maurel et al., 1999]. Activation of various subtypes of serotonin receptors, such as $5HT_{2A,}$ $5\text{-}HT_{2C}$, and $5\text{-}HT_{1B}$ reduce ethanol consumption [Maurel et al., 1999]. Clinical pharmacological agents increasing serotonergic activities were found to have a modest degree, but not consistent across studies, of action in reducing alcohol intake [Lejoyeux, 1996; Garbutt et al., 1999].

Low serotonergic function was responsible for poor impulse control, such as aggressiveness and self-injurious behaviors [Roy, 1990]. Alcohol-dependent individuals often show poor impulse control behavior [Linnolia et al., 1989]. There is a consistent finding that a proportion of alcoholism patients have association with behavior symptoms assigned as Type 2 alcoholism [Cloninger et al., 1981], Type B alcoholism [Barbor et al., 1992], and deviant-behavior type alcoholism [Hwu et al., 1992]. This behavior-disordered subtype of alcoholism is possibly based on the pathophysiological mechanism of reduced serotonergic functions.

Based on this background, a promising area of study is the search for genes related to serotonin transmission, responsible for vulnerability to alcoholism [Hill et al., 1999]. Serotonin transporter gene (HTT) polymorphism (HTTLPR) was not found to be associated with alcohol dependence [Edenberg et al., 1998]. The LL genotype of the HTT was found to be associated with a low level of alcohol response which was a vulnerability marker of alcoholism [Schuckit et al., 1999]. Nakamura et al. [1999] reported a positive association of alcohol dependence with a (A/G) polymorphism of the promoter region of 5HT2A receptor gene located at chromosome 13q14-21 [Hsieh et al., 1990]. The present study was designed with the hypothesis that there is an association between alcoholism with behavior problems and

Contract grant sponsor: the Department of Health, Taiwan; Contract grant number: DOH-85-TD-109; Contract grant sponsor: the National Health Research Institute, Taiwan; Contract grant numbers: DOH 87-HR-306, NHRI-GT-EX89P825P; Contract grant sponsor: National Taiwan University Hospital; Contract grant number: NTUH-88-A0015.

*Correspondence to: Hai-Gwo Hwu, M.D., Department of Psychiatry, National Taiwan University Hospital, No. 7 Chung-Shan S. Rd., Taipei, 100, Taiwan. E-mail: haigohwu@ha.mc.ntu.edu.tw

Received 29 November 1999; Accepted 26 April 2000

receptor gene T/C polymorphism, at position 102, of the serotonin receptor subtype 2A (HTR2A) [Hsieh et al., 1990; Warren et al., 1993].

METHODS AND SAMPLES

The sample of this study consisted of 85 subjects (45 men, 40 women) with alcohol abuse (AA), 75 subjects (51 men, 24 women) with alcohol dependence (AD), and 70 normal control subjects (21 men, 49 women), after obtaining informed consent. The diagnoses of alcohol abuse and alcohol dependence by DSM-III criteria [APA, 1980], were assessed using the alcoholism section of the Chinese modified version of the Diagnostic Interview Schedule (DIS-CM) [Hwu et al., 1984, 1986; Lin et al., 1995] with satisfactory interrater reliability. Eleven items of childhood–adolescent behavioral symptoms included in this study were repeated truancy, repeated fighting with fists, ever fighting with arms, running away from home, lying, stealing, damaging other's property, being cruel to animals, attacking others without notice, setting fires, and robbery. In this study, those subjects who had three or more items of behavioral symptoms were coded as "with a behavior problem."

RESULTS

Data were obtained by using personal interviews performed by well-trained research interviewers. Peripheral blood samples were drawn and the DNA of white blood cells was extracted using the method of Sambrook et al. [1989] with modifications. Genotyping of the T/C polymorphism at position 102 of HTR2A was done by polymerase chain reaction (PCR) [Warren et al., 1993; Arranz et al., 1995] using the sense primer of 5′-TCT GCT ACA AGT TCT GGC TT-3′, and the antisense primer of 5′-CTG CAG CTT TTT CTA GGG-3′. The 102T allele (T102) showed a PCR product fragment of 342 bp and the 102C allele (C102) showed two PCR product fragments of 126 bp and 216 bp after digestion with restriction enzyme of MspI.

The allele frequencies T102 and C102 in the AA, AD, and normal control groups were 72.9%, 69.3%, and 69.3%, and 27.1%, 30.7%, and 30.7%, respectively. The homozygous genotypes of T102/T102 and C102/C102 had frequencies of 50.6%, 44.0%, and 44.3%, and 4.7%, 5.3%, and 5.7% in the AA, AD, and normal control groups, respectively. No significant differences in genotype or allele distributions between study groups were found.

Table I shows the frequencies of genotypes and alleles in the three study groups with behavioral problems. The group of male alcohol abuse subjects with behavior problems was found to have lower frequencies of the genotype of homozygous T102 and to have higher frequencies of the genotype of homozygous C102 in contrast with the male groups of both alcohol dependence and normal control. The difference in frequencies of homozygous T102 genotype between the other two genotypes (T/C and C/C) between the male and female alcohol abuse subjects was statistically significant ($\chi^2 = 4.072$, df = 1, $P < 0.05$). The frequency of T102 was significantly lower in the male alcohol abuse subjects than in the female subjects with behavior problems ($\chi^2 = 4.187$, df = 1, $P < 0.05$).

Table II shows that the frequency of T102 in the male alcohol abuse group without behavioral problems was 79.5% and this was significantly higher ($\chi^2 = 4.328$, df = 1, $P < 0.05$) than that of the group with behavioral problems (59.1%). In the female alcohol abuse subjects, the frequencies of allele T102 in the groups with and without behavioral problems were similar (85.0% vs. 73.3%).

DISCUSSION AND CONCLUSION

This study used 11 behavior problem symptoms to evaluate the behavior problems in childhood and adolescence. The presence of a behavior problem was defined as having at least three symptom items. This is an arbitrary criterion for definition of having behavioral problems to indicate the tendency of having poor

TABLE I. Genotypes and Allele Frequencies of T/C 102 Polymorphism of the Receptor Gene of Serotonin Receptor 2A in Study Groups of Alcohol Abuse, Alcohol Dependence, and Normal Controls With Behavioral Problem

	Alcoholism with behavioral problem								
	Alcohol abuse			Alcohol dependence			Normal controls		
Genotypes	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
T102/T102	7[1]	7[1]	14	15	3	18	2	5	7
	(31.9)	(70.0)	(53.8)	(50.0)	(33.3)	(46.2)	(50.0)	(45.5)	(46.7)
T102/C102	12	3	15	13	6	19	2	5	7
	(54.5)	(30.0)	(46.9)	(43.3)	(66.7)	(48.7)	(50.0)	(45.5)	(46.7)
C102/C102	3	0	3	2	0	2	0	1	1
	(13.6)	(0.0)	(9.4)	(6.7)	(0.0)	(5.1)	(0.0)	(9.0)	(6.6)
Total	22	10	32	30	9	39	4	11	15
Alleles									
T102	26[2]	17[2]	43	43	12	55	6	15	21
	(59.1)	(85.0)	(67.2)	(71.7)	(66.7)	(70.5)	(75.0)	(68.2)	(70.0)
C102	18	3	21	17	6	23	2	7	9
	(40.9)	(15.0)	(32.8)	(28.3)	(33.3)	(29.5)	(25.0)	(31.8)	(30.0)
Total	44	20	64	60	18	78	8	22	30

[1]T102/T102 vs. (T102/C102 and C102/C102) by sex: $\chi^2 = 4.072$, df = 1, $P < 0.05$.
[2](T102 vs. C102) by sex: $\chi^2 = 4.187$, df = 1, $P < 0.05$.

TABLE II. Comparison of Genotype and Allele Frequencies of T/C 102 Polymorphism of the Receptor Gene of Serotonin Receptor 2A Between Study Groups With Behavior Problem (GWB) and Without Behavioral Problems (WOB) in Male and Female Alcohol Abuse Cases

	Alcohol abuse			
	Males		Females	
Genotypes	GWB(%)	GOB(%)	GWB(%)	GOB(%)
T102/T102	7 (31.9)	13 (59.1)	7 (70.0)	15 (50.0)
T102/C102	12 (54.5)	9 (40.9)	3 (30.3)	14 (46.7)
C102/C102	3 (13.6)	0 (0.0)	0 (0.0)	1 (3.3)
Total	22	22	10	30
Alleles				
T102	26 (59.1)	35 (79.5)	17 (85.0)	44 (73.3)
C102	18 (40.9)*	9 (20.5)*	3 (15.0)	16 (26.7)
Total	44	44	20	60

*$\chi^2 = 4.328$, df = 1, $P < 0.05$.

impulse control which might be related to serotonin dysregulation [Roy et al., 1990]. This study shows that there is a significant difference in the allele frequencies of T102 and C102 between male alcohol abuse subjects with and without behavioral problems. The polymorphism of T/C 102 does not involve change in the amino acids of the protein. The best speculation for the significance of T/C polymorphism at the molecular biological level in male alcohol abuse may involve the affected translation through the secondary structure and the stability of the mRNA which, in turn, may decrease the functional activity of this receptor in male subjects and cause impulsive behaviors associated with alcohol abuse.

The study results emphasize that alcohol abuse defined by DSM-III is heterogeneous in nature and can be differentiated by gender and by the presence or absence of behavior problems. Moreover, the significance of behavior problems in alcohol abuse and alcohol dependence was also different. The polymorphism of MAOA gene [Sullivan et al., 1990] was found to be associated with Type 2 alcoholism, mainly of alcohol dependence cases, defined by Cloninger et al. [1981]. These data support the idea that the diagnostic categories of alcohol abuse and dependence are heterogeneous nosologically. The location of the HTR2A is near the gene for esterase D [Hsieh et al., 1990], and it was found to be associated with alcoholism [Tanna et al., 1998]. However, this finding was not replicated in another study [Wesner et al., 1991]. This inconsistent finding might be due to different study samples. Moreover, the inconsistent findings of the therapeutic effect of serotonergic medications [Lejoyeux, 1996; Garbutt et al., 1999] in alcoholism could also be explained by the heterogeneity of alcoholism. Future studies of this kind may have to consider the presence of behavior problems and the different diagnostic categories of alcohol abuse and dependence.

REFERENCES

APA (American Psychiatric Association). 1980. Diagnostic and statistical manual, 3rd ed. Washington, DC: American Psychiatric Press.

Arranz M, Collier D, Sodhi M, Ball D, Roberts G, Price J, Sham P, Kerwin R. 1995. Association between clozapine response and allelic variation 5-HT2A receptor gene. Lancet 346:281–282.

Barbor TF, Hofmann M, Delboca FK, Hesselbrock V, Meyer RE, Dolinsky ZS, Rounsaville B. 1992. Types of alcoholism. I. Evidence for an empirically derived typology based on indicators of vulnerability and severity. Arch Gen Psychiatr 49:599–608.

Chen K, Yang W, Grimsby J, Shib JC. 1992. The human 5-HT2 receptor is encoded by a multiple intron-exon gene. Mol Brain Res 14:20–22.

Cloninger CR, Bohman M, Sigvardsson S. 1981. Inheritance of alcohol abuse: cross-fostering analysis of adopted men. Arch Gen Psychiatr 38:861–868.

Garbutt JC, West SL, Carey TS, Lohr KN, Crews FT. 1999. Pharmacological treatment of alcohol dependence: a review of the evidence. JAMA 281:1318–1325.

Hill EM, Stoltenberg SF, Burmeister M, Closser M, Zucker RA. 1999. Potential associations among genetic markers in the serotonergic system and the antisocial alcoholism subtype. Exp Clin Psychopharmacol 7: 103–121.

Hsieh CL, Bowcock AM, Farrer LA, Hebert JM, Huang KN, Cavalli-Storza LL, Julius D, Francke U. 1990. The serotonin receptor subtype 2 locus HTR2 is on human chromosome 13 near genes for esterase D and retinoblastoma-1 and on mouse chromosome 14. Somat Cell Mol Genet 16:567–574.

Hwu HG, Yeh EK, Chang LY, Chen CT, Chen CC, Chen TY. 1984. The Chinese modification of NIMH-Diagnostic Interview Schedule-reliability study on assessment of psychiatric symptoms. Psychol Test 31:15–26.

Hwu HG, Yeh EK, Chang LY. 1986. Chinese diagnostic interview schedule. I. Agreement with psychiatrist's diagnosis. Acta Psychiatr Scand 73: 225–233.

Hwu HG, Chen CC, Yeh LL. 1992. A typology of alcoholism in Taiwan aborigines: a study on evolving process of community cases. Psychopathology 25:311–318.

Le Marquand D, Pihl RO, Benkelfat C. 1994. Serotonin and alcohol intake, abuse, and dependence: findings of animal studies. Biol Psychiatr 36: 395–421.

Lejoyeux M. 1996. Use of serotonin reuptake inhibitors in the treatment of alcoholism. Alcohol Alcohol 31(Suppl 1):69–75.

Lin LS, Hwu HG, Soong WT, Yeh LL, Chang C, Chang SH, Lin ST, Wang JD, Hsu MT, Chen CC. 1995. The establishment of an interview schedule for psychopathology of alcoholism: inter-rater reliability and procedure validity. Chin Psychiatr 9:111–121.

Linnolia M, De Jong J, Virkkunen M. 1989. Family history of alcoholism in violent offenders and impulsive fire setters. Arch Gen Psychiatr 46: 613–616.

Maurel S, De Vry J, De Beun R, Schreiber R. 1999. 5-HT_{2A} and 5-HT_{2C}/5-HT_{1B} receptors are differentially involved in alcohol preference and consummatory behavior in cAA rats. Psychopharm Biochem Behav 62: 89–96.

Meltzer HY. 1990. Role of serotonin in depression. Ann NY Acad Sci 600: 486–500.

Nakamura T, Matsushita S, Nishiguchi N, Kimura M, Yoshino A, Higuchi S. 1999. Association of a polymorphism of the 5HT2A receptor gene promoter region with alcohol dependence. Mol Psychiatr 4:85–88.

Roy A, Virkkunen M, Linnoila M. 1990. Serotonin in suicide, violence and alcoholism. In: Coccaro EF, Murphy DL, editors. Serotonin in major psychiatric disorders. Washington, DC: American Psychiatric Press. p 187–208.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual, 2nd ed. New York: Cold Spring Harbor Laboratory Press. p 914–919.

Schuckit MA, Mazzanti C, Smith TL, Ahmed U, Radel M, Iwata N, Goldman D. 1999. Selective genotyping for the role of 5-HT2A, 5-HT2C, and GABAa6 receptors and the serotonin transporter in the level of response to alcohol: a pilot study. Biol Psychiatr 45:647–651.

Sigvardsson S, Bohman M, Cloninger CR. 1996. Replication of the Stockholm adoption study of alcoholism. Arch Gen Psychiatr 53:681–687.

Sullivan JL, Baenziger JC, Wagner DL, Rauscher FP, Nurnberger JI Jr, Holmes JS. 1990. Platelet MAO in subtypes of alcoholism. Biol Psychiatr 27:911–922.

Tanna VL, Wilson AF, Winokur G, Elston RC. 1988. Possible linkage between alcoholism and esterase D. J Study Alcohol 4:472–476.

Virkkunen M, Linnoila M. 1997. Serotonin in early-onset alcoholism. Recent Dev Alcohol 13:173–189.

Warren JT Jr, Peacock ML, Rodriguez LC, Fink JK. 1993. An MspI polymorphism in the human serotonin receptor gene (HTR2A): detection by DGGE and RFLP analysis. Hum Mol Genet 2:338.

Wesner RB, Tanna VL, Palmer PJ, Thompson RJ, Growe RR, Wonokor G. 1991. Close linkage of esterase-D to unipolar depression and alcoholism is ruled out in eight pedigree. J Study Alcohol 52:609–612.