Association Between 5HT1b Receptor Gene and Methamphetamine Dependence

H. Ujike1,2,*, M. Kishimoto1, Y. Okahisa1, M. Kodama1, M. Takaki1, T. Inada2,3, N. Uchimura2,4, M. Yamada2,5, N. Iwata2,6, M. Iyo2,7, I. Sora2,8 and N. Ozaki2,9

1Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; 2JGIDA (Japanese Genetics Initiative for Drug Abuse), Japan; 3Seiwa Hospital, Tokyo, Japan; 4Department of Psychiatry, Kurume University Graduate School of Medicine, Kurume, Japan; 5Department of Psychogeriatrics, National Institute of Mental Health, National Center of Neurology and Psychiatry, Kodaira, Japan; 6Department of Psychiatry, Fujita Health University School of Medicine, Houmei, Japan; 7Department of Psychiatry, Chiba University Graduate School of Medicine, Chiba, Japan; 8Department of Neuroscience, Division of Psychobiology, Tohoku University Graduate School of Medicine, Sendai, Japan; 9Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan

Abstract: Several lines of evidence implicate serotonergic dysfunction in diverse psychiatric disorders including anxiety, depression, and drug abuse. Mice with a knock-out of the 5HT1b receptor gene (HTR1B) displayed increased locomotor response to cocaine and elevated motivation to self-administer cocaine and alcohol. Previous genetic studies showed significant associations of HTR1B with alcohol dependence and substance abuse, but were followed by inconsistent results. We examined a case-control genetic association study of HTR1B with methamphetamine-dependence patients in a Japanese population. The subjects were 231 patients with methamphetamine dependence, 214 of whom had a comorbidity of methamphetamine psychosis, and 248 age- and sex-matched healthy controls. The three single nucleotide polymorphisms (SNPs), rs130058 (A-165T), rs1228814 (A-700C) and rs1228814 (A+1180G) of HTR1B were genotyped. There was no significant difference in allelic and genotypic distributions of the SNPs between methamphetamine dependence and the control. Genetic associations of HTR1B were tested with several clinical phenotypes of methamphetamine dependence and/or psychosis, such as age at first abuse, duration of latency from the first abuse to onset of psychosis, prognosis of psychosis after therapy, and complication of spontaneous relapse of psychotic state. There was, however, no association between any SNP and the clinical phenotypes. Haplotype analyses showed the three SNPs examined were within linkage disequilibrium, which implied that the three SNPs covered the whole HTR1B, and distribution of estimated haplotype frequency was not different between the groups. The present findings may indicate that HTR1B does not play a major role in individual susceptibility to methamphetamine dependence or development of methamphetamine-induced psychosis.

Keywords: Methamphetamine dependence, association study, HTR1B, haplotype.

INTRODUCTION

Family and twin studies have provided evidence that genetic factors can influence individual differences in vulnerability to substance abuse and dependence [1, 2]. We previously reported that patients with methamphetamine use disorders showed substantial individual differences in psychotomimetic and psychotogenic effects of methamphetamine consumption, e.g., intensity of subjective euphoric effects, latency to onset of methamphetamine-induced psychosis, and prognosis of psychosis after discontinuance of methamphetamine use [3], whose clinical variations should be affected by individual genetic background.

Pharmacological manipulation of serotonergic signaling can modulate the activity of brain reward pathways, and thus the effects of substance dependence to diverse classes of drugs. Fluoxetine, a selective serotonin uptake inhibitor, reduced self-administration of cocaine [4]. Ethanol intake decreased after the administration of 5-HT precursors, 5-HT uptake inhibitors, intracerebral 5-HT, and postsynaptic 5-HT agonists in animals and humans [5]. These serotonergic effects against drug abuse could be mediated by 5HT1b receptors at least. Administration of a 5HT1b agonist, CP-94,253, reduced ethanol self-administration and alcohol-induced aggressive behaviors via activation of postsynaptic 5HT1b receptors [6, 7]; in contrast, it facilitated cocaine reward by reducing 5HT release via 5HT1b autoreceptor stimulation at presynaptic sites [8]. Mice lacking 5HT1b receptors displayed increased locomotor response to and self-administration of cocaine [9], and elevated alcohol consumption [10].

Previous genetic studies indicated that the 5HT1b receptor gene (HTR1B, MIM 182131) was associated with drug dependence and related behaviors. Thus, HTR1B polymorphisms were reported to be associated significantly with al-
cohabitation with antisocial behaviors [11, 12], whole alcoholism [13, 14], substance dependence [15] and heroin addiction [16], although there were also several inconsistent reports [17, 18]. Therefore, in order to investigate the roles of \textit{HTR1B} in substance dependence, we examined a possible genetic association of \textit{HTR1B} with methamphetamine dependence in a Japanese population.

\section*{METHODS}

\subsection*{Subjects}

The subjects consisted of 231 patients with methamphetamine dependence (184 male, 47 female; mean age±SD, 36.6±11.8) and 248 age-, sex-, and geographical origin-matched healthy controls (198 male, 50 female; mean age±SD, 36.6±10.6), who have no individual or family history of drug dependence or major psychotic disorders such as schizophrenia and bipolar disorders. Almost all patients (N=214) are or were co-morbid with methamphetamine-induced psychosis. All subjects were unrelated Japanese. Consensus diagnoses of methamphetamine dependence were made by two trained psychiatrists according to the ICD-10 criteria on the basis of interviews and medical records. The study protocol and purpose were explained to all subjects participating in the study, and written informed consent was obtained from all subjects. This study was approved by the Ethics Committee of each participating institute of Japanese Genetics Initiative for Drug Abuse (JGIDA) [19].

The patients with methamphetamine dependence and/or psychosis were divided into subgroups according to several clinical phenotypes that may indicate indirectly the severity of and liability to dependence and psychosis: 1) age at first abuse of methamphetamine: younger than 20 years old, which is underage in Japan, or older; 2) latency to onset of psychotic state after initial methamphetamine consumption: divided into two groups by median latency of 3 years; 3) Duration of psychotic state after discontinuation of use and therapy with antipsychotics: transient type and prolonged type, which were defined as psychosis that subsides within one month or lasts longer than one month, respectively; 4) complication of spontaneous psychosis after remission of methamphetamine-induced psychosis, and 5) multi-substance abuse status.

\subsection*{Genotyping}

\textit{HTR1B} consists of single exon and is a relatively small gene of about 11.7 Kb. HapMap data indicates that there is only one single nucleotide polymorphism (SNP) in the exon, rs6297, which was proven polymorphic in a Japanese population. However, we did not examine this SNP because it is synonymous, Val3Val, indicating no or less physiological involvement. Instead, we genotyped three SNPs flanking the gene, rs6297 (A-700C) and rs130058 (A-161T) in the 5' flanking region and rs1228814 (A+1180G) in the 3' flanking region, which have potential to be functional. Genotyping was performed by the PCR-RFLP method. The genomic DNA was extracted from peripheral leukocytes using a standard method. Each polymorphic site was amplified by PCR (the PCR primer sequence of each SNP is available on request) in a 15-ml volume containing 3% dimethyl sulfoxide and 0.75 units of Taq DNA polymerase (Promega Co., Japan) using a unique primer set. The PCR reaction was performed under the following conditions: 95°C for 5 min, then 35 cycles of 30 s of denaturing at 95°C, 1 min of annealing at the appropriate temperature, and 30 s of extension, and final elongation at 72°C for 10 min. The PCR products were digested with the corresponding restriction enzyme for each polymorphism, NdeI for rs6297, NlaIII for rs130058 and CfoI13f for rs1228814, and then electrophoresed on 3.0% agarose gels and stained with GelStar (Takara Co., Japan). All genotyping was performed in a blinded fashion, with the control and cases samples mixed randomly. The genotyping of the SNPs were confirmed in part by direct sequencing.

\section*{Statistical Analysis}

Statistical analysis of association was performed using SNPAlvize software (Dynacom Co., Japan). Deviation from Hardy-Weinberg equilibrium and the case-control study were tested using the \textit{\chi}^2 test for goodness of fit and \textit{\chi}^2 test for dependence, respectively. Linkage disequilibrium (LD) was tested using the \textit{\chi}^2 test, and D' and \textit{r}^2 values were made the index in the authorization of LD. Case-control haplotype analysis was performed by the permutation method, and permutation \textit{p}-values were calculated based on 100,000 replications.

\section*{RESULTS}

The genotype distribution and allele frequencies of the each polymorphism are shown in Table 1. The genotype distributions of patients and control subjects did not deviate from Hardy-Weinberg equilibrium at any SNP examined. We found no significant difference between the patients and controls in the frequencies of the genotype or allele at any SNP of \textit{HTR1B} (rs6297: allele: \textit{p}=0.37, genotype: \textit{p}=0.38, rs130058: allele: \textit{p}=0.30, genotype: \textit{p}=0.33 rs1228814: allele: \textit{p}=0.14, genotype: \textit{p}=0.47).

We estimated the pairwise LD between the three SNPs of \textit{HTR1B} using the D' and \textit{r}^2 values as an index. A D' of more than 0.7 was found between all the SNPs (0.8455 between rs6297 and rs130058, 1.000 between rs6297 and rs1228814, 0.8216 between rs130058 and rs1228814) indicating that the three SNPs are in linkage disequilibrium (LD) and located within one LD block. Then, we performed case-control haplotype analysis (Table 2). There were 5 kinds of haplotypes consisting of the three SNPs. There was no significant difference in distribution of haplotype between methamphetamine dependence and controls (overall permutation \textit{p}=0.81). Neither haplotype consisting of the two SNPs in the promoter region (rs6297 and rs130058) showed a significant difference in distribution between the groups.

Additional analyses of subgroups of patients with methamphetamine dependence/psychosis stratified by five items of clinical phenotypes (Table 3) revealed that there was no significant association of any SNP of \textit{HTR1B} with any clinical phenotype of methamphetamine dependence and/or psychosis.

\section*{DISCUSSION}

The 5HT1b receptors are expressed in the brain of rodents, and homologous 5HT1D receptors are expressed in the human brain. The 5HT1b receptors are located at nerve
terminals of various pathways and act as autoreceptors that are involved in the regulation of release of diverse neurotransmitters, including serotonin itself [20]. The 5HT1b receptors are also located at postsynaptic sites. A lot of studies suggest that 5HT1b receptors are implicated in several physiological functions, behaviors, and neuropsychiatric disorders including migraine, aggression, anxiety, depression, and substance dependence [20].

Genetic associations of HTR1B have been examined with various psychiatric conditions such as antisocial behaviors, suicide, depression, and schizophrenia. As to substance dependence, Lappapleinene et al. [11] found that rs6296, a synonymous SNP in exon 1 (G861C, Val3Val), was associated with antisocial alcoholism in two independent populations of alcoholic patients for the first time, but this was followed by consistent [12] and inconsistent results [15, 17, 18, 21]. Finally, Fehr et al. [13] reported that the risk allele of 861C reported by Lappapleinene et al. [11] was protective in their patients with alcoholism. These inconsistencies among alcoholism studies may indicate that status of co-morbidity with other substance abuse could influence the results because HTR1B was shown to be associated with substance abuse [15] and heroin addiction [16].

In the present study, we examined three SNPs in the 5’ and 3’ flanking regions of HTR1B, rs6297 (A-700C), rs130058 (A-161T), and rs1228814 (A+1180G) in patients with methamphetamine dependence and found no association at any loci. Neither was any association found with several clinical phenotypes, such as initial abuse of methamphetamine at a younger age, rapid onset of psychotic state induced by methamphetamine, longer duration of psychosis after discontinuance of methamphetamine abuse, complication of spontaneous relapse of psychosis after remission, and multi-substance abuse status. Haplotype analysis of the three SNPs also showed no significant difference in haplotype distribution between the patients and controls. As the LD block consisting of the three SNPs covers the whole of HTR1B, it is unlikely that any untested polymorphism including G861C in HTR1B could be associated with methamphetamine dependence or its clinical phenotypes. Our findings are consistent with a study of cocaine, another psychostimulant, which showed that T-261G, A-161T, and G861C of HTR1B was not associated with cocaine abuse [17].

Duan et al. [22] examined effects of common SNPs in the promoter region of HTR1B on its transcription activity by in vitro reporter assay and revealed that T-261G and A-161T (rs130058) potently affected gene expression. The haplotypes consisting of -261G and -161A enhanced transcriptional activity 2.3-fold compared with major haplotype consisting of -261T and -161T. The A-161T polymorphism altered characteristics of binding to AP-1 transcription factor.  

| Loci | Groups          | N  | Genotype (%) | p   | Allele (%) | p   |
|------|-----------------|----|--------------|-----|------------|-----|
|      |                 |    | A/A          | A/G | G/G        | A/G |
| SNP1 | Control         | 248| 73.4         | 23.8| 2.8        | 85.3| 14.7|
|      | MAP-dependence  | 228| 68.9         | 27.6| 3.5        | 0.37| 82.7| 17.3| 0.38|
| SNP2 | Control         | 227| 87.2         | 12.8| 0          | 93.6| 6.4 |
|      | MAP-dependence  | 229| 89.5         | 10.5| 0          | 0.3  | 94.8| 5.2 | 0.33|
| SNP3 | Control         | 246| 73.6         | 25.2| 1.2        | 86.2| 13.8|
|      | MAP-dependence  | 225| 70.7         | 27.5| 1.8        | 0.14 | 84.4| 15.6| 0.47|

Global permutation p value =0.81 (c²=3.20).

In Table 1, Case-Control Association Analyses of HTR1B

In Table 2, Haplotype Analysis of HTR1B in Methamphetamine Dependence

| Haplotype | Controls | MAP-Dependence | p   |
|-----------|----------|----------------|-----|
| A-T-C     | 0.6973   | 0.6721         | 0.41|
| G-T-C     | 0.1480   | 0.1673         | 0.43|
| A-T-A     | 0.0902   | 0.1095         | 0.33|
| A-A-A     | 0.0520   | 0.0461         | 0.68|
| A-A-C     | 0.0094   | 0.0034         | 0.26|
### Table 3. Association of \textit{HTR1B} with Clinical Phenotypes of Methamphetamine Dependence and Psychosis

| SNP1 (rs6297) | N | Genotype (%) | p | Allele (%) | p |
|---------------|---|--------------|---|------------|---|
|               |   | A/A          |   |            |   |
| Age at first use |   |              |   |            |   |
| 20y <=        | 111 | 0.68 | 0.26 | 0.05 | 0.82 | 0.18 |
| 19y >=        | 113 | 0.68 | 0.30 | 0.02 | 0.37 | 0.83 | 0.17 | 0.38 |
| Latency of psychosis |   |              |   |            |   |
| 3y >         | 103 | 0.64 | 0.31 | 0.05 | 0.80 | 0.20 |
| 3y <=        | 83  | 0.70 | 0.28 | 0.02 | 0.57 | 0.84 | 0.16 | 0.31 |
| Prognosis of psychosis |   |              |   |            |   |
| Transient    | 114 | 0.68 | 0.30 | 0.03 | 0.82 | 0.18 |
| Prolonged    | 84  | 0.69 | 0.26 | 0.05 | 0.65 | 0.82 | 0.36 | 0.94 |
| Spontaneous relapse of psychotic symptoms |   |              |   |            |   |
| +            | 84  | 0.62 | 0.36 | 0.02 | 0.80 | 0.20 |
| −            | 129 | 0.71 | 0.24 | 0.05 | 0.15 | 0.83 | 0.17 | 0.35 |
| Poly-substance abuse |   |              |   |            |   |
| +            | 158 | 0.69 | 0.28 | 0.03 | 0.83 | 0.17 |
| −            | 63  | 0.68 | 0.27 | 0.05 | 0.85 | 0.82 | 0.18 | 0.77 |
| SNP2 (rs130058) | N | Genotype (%) | p | Allele (%) | p |
|               |   | T/T          |   |            |   |
| Age at first use |   |              |   |            |   |
| 20y <=        | 111 | 0.86 | 0.14 | 0.00 | 0.93 | 0.07 |
| 19y >=        | 114 | 0.92 | 0.08 | 0.00 | 0.31 | 0.96 | 0.04 | 0.33 |
| Latency of psychosis |   |              |   |            |   |
| 3y >         | 103 | 0.91 | 0.09 | 0.00 | 0.96 | 0.04 |
| 3y <=        | 84  | 0.90 | 0.10 | 0.00 | 0.85 | 0.95 | 0.05 | 0.86 |
| Prognosis of psychosis |   |              |   |            |   |
| Transient    | 115 | 0.88 | 0.12 | 0.00 | 0.94 | 0.06 |
| Prolonged    | 84  | 0.93 | 0.07 | 0.00 | 0.24 | 0.96 | 0.04 | 0.26 |
| Spontaneous relapse of psychotic symptoms |   |              |   |            |   |
| +            | 85  | 0.92 | 0.08 | 0.00 | 0.96 | 0.04 |
| −            | 129 | 0.87 | 0.13 | 0.00 | 0.26 | 0.93 | 0.07 | 0.28 |
| Poly-substance abuse |   |              |   |            |   |
| +            | 159 | 0.90 | 0.10 | 0.00 | 0.95 | 0.05 |
| −            | 63  | 0.87 | 0.13 | 0.00 | 0.57 | 0.94 | 0.06 | 0.58 |
Therefore, our negative findings may be significant and indicate that higher or lower density of the 5HT1b receptor due possession of -161A or -161T of HTR1B does not affect individual susceptibility to methamphetamine dependence and psychosis.

ACKNOWLEDGEMENTS
This work was partly supported by the Zikei Institute of Psychiatry (Okayama, Japan) and Grants-in-Aid from the Japanese Ministry of Health, Labor and Welfare.

REFERENCES
[1] Crabbe, J.C.; Belknap, J.K.; Buck, K.J. Genetic animal models of alcohol and drug abuse. Science, 1994, 264, 1715-1723.
[2] Koob, G.F. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol. Sci., 1992, 13, 177-184.
[3] Ujike, H.; Sato, M. Clinical features of sensitization to methamphetamine observed in patients with methamphetamine dependence and psychosis. Ann. N. Y. Acad. Sci., 2004, 1025, 279-287.
[4] Carroll, M.E.; Lac, S.T.; Asencio, M.; Kragh, R. Fluoxetine reduces intravenous cocaine self-administration in rats. Pharmacol. Biochem. Behav., 1990, 35, 237-244.
[5] Naranjo, C.A.; Sellers, E.M.; Lawrin, M.O. Modulation of ethanol intake by serotonin uptake inhibitors. J. Clin. Psychiatry, 1986, 47 (Suppl), 16-22.
[6] Maurel, S.; De Vry, J.; Schreiber, R. 5-HT receptor ligands differentially affect operant oral self-administration of ethanol in the rat. Eur. J. Pharmacol., 1999, 370, 217-223.
[7] Fish, E.W.; Faccidomo, S.; Miczek, K.A. Aggression heightened by alcohol or social instigation in mice: reduction by the 5-HT(1B) receptor agonist CP-94,253. Psychopharmacology (Berl), 1999, 146, 391-399.
[8] Parsons, L.H.; Weiss, F.; Koob, G.F. Serotonin1B receptor stimulation enhances cocaine reinforcement. J. Neurosci., 1998, 18, 10078-10089.
[9] Rocha, B.A.; Scearce-Levie, K.; Lucas, J.J.; Hiroi, N.; Castanon, N.; Crabbe, J.C.; Nestler, E.J.; Hen, R. Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature, 1998, 393, 175-178.
[10] Crabbe, J.C.; Phillips, T.J.; Feller, D.J.; Hen, R.; Wenger, C.D.; Lessov, C.N.; Schafer, G.L. Elevated alcohol consumption in null mutant mice lacking 5-HT1B serotonin receptors. Nat. Genet., 1996, 14, 98-101.
[11] Lappalainen, J.; Long, J.C.; Eggert, M.; Ozaki, N.; Robin, R.W.; Brown, G.L.; Naukkarinen, H.; Virkkunen, M.; Linnoila, M.; Goldman, D. Linkage of antisocial alcoholism to the serotonin 5-HT1B receptor gene in 2 populations. Arch. Gen. Psychiatry, 1998, 55, 989-994.
[12] Soyka, M.; Preuss, U.W.; Koller, G.; Zill, P.; Bondy, B. Association of 5-HT1B receptor gene and antisocial behavior in alcoholism. J. Neural. Transm., 2004, 111, 101-109.
[13] Fehr, C.; Grintschuk, N.; Szegedi, A.; Anghelescu, I.; Klawe, C.; Singer, P.; Hiemke, C.; Dahmen, N. The HTR1B 861G>C receptor polymorphism among patients suffering from alcoholism, major depression, anxiety disorders and narcolepsy. Psychiatry Res., 2000, 97, 1-10.
[14] Sun, H.F.; Chang, Y.T.; Fann, C.S.; Chang, C.J.; Chen, Y.H.; Hsu, Y.P.; Yu, W.Y.; Cheng, A.T. Association study of novel human serotonin 5-HT(1B) polymorphisms with alcohol dependence in Taiwanese. Hum. Biol. Psychiatry, 2002, 51, 896-901.
[15] Huang, Y.Y.; Oquendo, M.A.; Friedman, J.M.; Greenhill, L.L.; Brodsky, B.; Malone, K.M.; Khait, V.; Mann, J.J. Substance abuse disorder and major depression are associated with the human 5-HT1B receptor gene (HTR1B) G861C polymorphism. Neuropsychopharmacology, 2003, 28, 163-169.
[16] Proudnikov, D.; LaForge, K.S.; Hofflich, H.; Levensien, M.; Gordon, D.; Barral, S.; Ott, J.; Kreek, M.J. Association analysis of

| SNP3 (rs1228814) | N   | Genotype (%) | p   | Allele (%) | p   |
|-----------------|-----|--------------|-----|------------|-----|
|                 |     | C/C          | C/A | A/A        |     |
| Age at first use| 20y <= | 110 | 0.72 | 0.25 | 0.04 | 0.84 | 0.16 |
|                 | 19y >= | 111 | 0.68 | 0.32 | 0.00 | 0.14 | 0.84 | 0.16 | 0.47 |
| Latency of psychosis | 3y > | 100 | 0.76 | 0.22 | 0.02 | 0.87 | 0.13 |
|                 | 3y <= | 83  | 0.66 | 0.33 | 0.01 | 0.27 | 0.83 | 0.17 | 0.23 |
| Prognosis of psychosis | Transient | 113 | 0.71 | 0.28 | 0.01 | 0.85 | 0.15 |
|                 | Prolonged | 82  | 0.73 | 0.24 | 0.02 | 0.59 | 0.85 | 0.15 | 0.91 |
| Spontaneous relapse of psychotic symptoms | + | 81  | 0.73 | 0.27 | 0.00 | 0.86 | 0.14 |
|                 | - | 129 | 0.69 | 0.28 | 0.03 | 0.27 | 0.83 | 0.17 | 0.34 |
| Poly-substance abuse | + | 156 | 0.70 | 0.28 | 0.02 | 0.84 | 0.16 |
|                 | - | 62  | 0.69 | 0.29 | 0.02 | 0.98 | 0.84 | 0.16 | 0.98 |
polymorphisms in serotonin 1B receptor (HTR1B) gene with heroin addiction: a comparison of molecular and statistically estimated haplotypes. Pharmacogenet. Genom., 2006, 16, 25-36.

[17] Cigler, T.; LaForge, K.S.; McHugh, P.F.; Kapadia, S.U.; Leal, S.M.; Kreek, M.J. Novel and previously reported single-nucleotide polymorphisms in the human 5-HT(1B) receptor gene: no association with cocaine or alcohol abuse or dependence. Am. J. Med. Genet., 2001, 105, 489-497.

[18] Kranzler, H.R.; Hernandez-Avila, C.A.; Gelernter, J. Polymorphism of the 5-HT1B receptor gene (HTR1B): strong within-locus linkage disequilibrium without association to antisocial substance dependence. Neuropsychopharmacology, 2002, 26, 115-122.

[19] Ujike, H.; Harano, M.; Inada, T.; Yamada, M.; Komiyama, T.; Sekine, Y.; Sora, I.; Iyo, M.; Katsu, T.; Nomura, A.; Nakata, K.; Ozaki, N. Nine- or fewer repeat alleles in VNTR polymorphism of the dopamine transporter gene is a strong risk factor for prolonged methamphetamine psychosis. Pharmacogenomics J., 2003, 3, 242-247.

[20] Sari, Y. Serotonin 1B receptors: from protein to physiological function and behavior. Neurosci. Biobehav. Rev., 2004, 28, 565-582.

[21] Sinha, R.; Cloninger, C.R.; Parsian, A. Linkage disequilibrium and haplotype analysis between serotonin receptor 1B gene variations and subtypes of alcoholism. Am. J. Med. Genet. B Neuropsychiatr. Genet., 2003, 121B, 83-88.

[22] Duan, J.; Sanders, A.R.; Molen, J.E.; Martinolich, L.; Mowry, B.J.; Levinson, D.F.; Crowe, R.R.; Silverman, J.M.; Gejman, P.V. Polymorphisms in the 5'-untranslated region of the human serotonin receptor 1B (HTR1B) gene affect gene expression. Mol. Psychiatry, 2003, 8, 901-910.