IJHG 2 (1): 11-14 (2002)
PRINT: ISSN 0972-3757 ONLINE: 2456-6360 **DOI: 10.31901/24566330.2002/02.01.03**

Functional Polymorphism ($Ser^{311} \rightarrow Cys^{311}$) in the Dopamine D2 Receptor Gene and Alcohol Drinking Habits in Siberia

A. Belkovets[1], S. Kurilovich[1] and D. P. Agarwal[2]

[1]Institute of Internal Medicine, Novosibirsk, Russia
[2]Institute of Human Genetics, University of Hamburg, 22529 Hamburg, Germany

KEY WORDS DRD2; genetic polymorphism; alcohol drinking habits; Siberia

ABSTRACT The human dopamine receptor D2 gene is an important candidate gene for drug addiction, alcoholism and/or for the modification of its severity. In the present study, the distribution of DRD2 polymorphism (Ser^{311}/Cys^{311}) among a random Siberian female population sample was examnined and correlated with self-reported alcohol drinking and alcohol-induced flushing response. The Cys^{311} variant was detected by DNA amplification using PCR followed by digestion with the restriction enzyme Sau961. The Ser^{311}/Cys^{311} heterozygous genotype was present in 4% of the samples and the frequency of the Cys^{311}-encoding allele was found to be 0.021. Subjects with Cy^{311}/Cys^{311} allele consumed alcohol less frequently than subjects with Ser^{311}/Cys^{311} allele.

INTRODUCTION

The human dopamin 2 receptor gene (DRD2) is one of the candidate genes possibly contributing to the genetic vulnerability for several neuropsychiatric diseases, including schizophrenia, alcoholism and/or drug abuse (Blum et al. 1995; Noble 2000; Finckh 2001; Lu et al. 2001). Restriction of DRD2 by TaqI yields polymorphic fragment lengths identifying alleles A1 and A2 which differ in average amounts of receptor sites. Since the first report by Blum et al. (1990) suggesting an association of D2 dopamine receptor gene and alcoholism, the possible role of DRD2 receptor locus in the etiology of alcoholism has been the focus of considerable attention (Pato et al. 1993; Kidd et al. 1996; Noble 2000). However, no evidence of linkage of Al allele and increased susceptibility to alcoholism in familiy-based and association studies has been found (Gelernter and Kranzler 1999; Sander et al. 1999; Blomqvist et al. 2000; Parisian et al. 2000). More recent reports, nevertheless, do hint to a possible linkage of the dopamine receptor gene with medical complications of alcoholism (Lawford et al. 1997). Moreover, polymorphism of the D2 dopamine receptor gene seems to be an important determinant of P300 latency (Nacher 2000). The observed association of the alleles of the human D2 dopamine receptor locus may not possibly be due to linkage with a gene for alcoholism, but could be a cause of the progression of the disease in individuals genetically predisposed to alcoholism.

In addition to the widely distributed TaqI polymorphism (Kidd et al. 1998), the human dopamine D2 receptor gene has three other polymorphic variants that predict the amino acid substitutions Val96→Ala, Pro310→Ser, and Ser311→Cys in the receptor protein. The C→G polymorphism in exon 7 of the D2 receptor gene leads to Ser^{311}/Cys^{311} mutation (Itokawa et al. 1993). The reported frequencies of the Ser^{311}/Cys^{311} variant allele vary from 0.019 to 0.035 in Japanese (Itokawa et al. 1993; Arinami et al. 1994; Higuchi et al. 1994; Nanko et al. 1994), and from 0.008 to 0.018 in Caucasian healthy subjects (Gejman et al. 1994; Sobell et al. 1994). An association of the structural Ser^{311}/Cys^{311} variant with alcoholism was reported in a large Japanese sample (Higuchi et al. 1994). Moreover, an association of the DRD2 Ser^{311}/Cys^{311} variant with schizophrenia has been reported (Arinami et al. 1994; Kaneshima et al. 1997; Serretti et al. 1998). However, data from other investigators do not support an association of Ser^{311}/Cys^{311} variant with schizophrenia and / or alcoholism (Chen et al. 1996; Crawford et al. 1996; Finckh et al. 1996; Tanaka et al. 1996; Verga et al. 1997; Goldman et al. 1998; Spurlock et al. 1998).

In the present study we have examined the incidence of Ser^{311}/Cys^{311} mutation among a Siberian female population sample from Novosibirsk (n=299). The frequency of the variant allele was correlated with the self-reported alcohol consumption and the incidence of alcohol-induced flushing response.

Mailing address: Dr. Dharam P. Agarwal, Ph.D., D.Sc., Professor of Human Genetics, Institute of Human Genetics, University of Hamburg, Butenfeld 42, 22529 Hamburg, *Phone:* (+4940) 42803 4586 *Fax:* (+4940) 42803 3122, *Email:* agarwal@uke.uni-hamburg.de

MATERIALS AND METHODS

The study was carried out on blood samples collected under the WHO multinational program MONICA ("Monitoring of Trend and Determinants in Cardiovascular Diseases"). lt is a larger project that includes an investigation of cardiovascular disorders, diabetes mellitus, anemias, alcohol consumption and other medical anomalies. A total of 875 women (age = 25-65 yrs) were investigated, using confidential interviews with the help of a questionnaire and medical examrnations. Venous blood was obtained for biochemical and genetic analysis from 299 females DNA samples were prepared from clotted blood using conventional methods (treatment with proteinase K, phenol-chloroform extraction and ethanol precipitation).

For DNA amplification the following primers (5'-CCAGCTGACTCTCCCCGACCGGT-3') and (5'-TTGGGCATGGTCTGGATCTCAAA-3') were used as forward and reverse primers, respectively. Twenty five microliters of the PCR reaction mixture contained 0.5 µl of 10 mM solution of dNTP; 2.5 µl of PCR buffer; 0.5 µl of 100 pM solution of each primer; 0.8 µl of 50 mM $MgC1_2$; 19 µl H_20; 1 µl DNA and 1 unit of Taq polymerase. Amplification was performed in the following mode: 1 cycle 4 min at 95 °C, 2 min at 65 °C; 35 cycles, 30 sec at 95 °C, 50 sec at 65°C, 10 sec at 72 °C; 1 cycle, 10 min at 72 °C.

Eight microliters of PCR product was digested with the restriction enzyme Sau969 for overnight. The DNA bands were separated on 8% polyacrylamide gel and visualized by silver staining. Amplification product size was 112 bp for the homozygous Ser^{311}/Ser^{311} genotype, 112 and 133 bp for the heterozygous Ser^{311}/Cys^{311} genotype and 133 bp for the homozygous Cys^{311}/Cys^{311} genotype.

RESULTS AND DISCUSSION

A number of studies have been published concerning alcohol drinking pattern in Russian populations (Bobak et al. 1999; Nemtsov 2000). While in many studies a relationship between alcohol metabolism genes and alcohol consumption has been investigated (Kurilovich et al. 1998; Belkovets et al. 2001), only a few studies have reported on the polymorphism of DRD2 gene in Russian populations (Avksentyuk et al. 1995; Galeeva et al. 2000). The major objective of the present study was to document drinking patterns and flushing response in relation to DRD2 receptor gene polymorphism among the female urban white population living in Novosibirsk.

Genotype and Allele Frequency Distribution

The distribution of the DRD2 genotypes among Novosibirsk sample is presented in Table 1. Cys^{311} variant allele was found to be 0.021, quite similar to that found in other Caucasian populations (0.008-0.018) (Gejman et al. 1994; Sobell et al. 1994; Craddock et al. 1995).

The distribution of DRD2 genotypes in groups with different frequency of alcohol consumption are presented in table 2.

While the common Ser^{311}/Ser^{311} homozygous genotype was found in all the three alcohol drinking groups, the mutant allele was found only in group 2 and group 3 subjects. However, neither the genotype nor the allele frequency distribution showd a statistically significant difference between any of the three groups.

Table 1: Distribution of DRD2 genotypes and allele frequency in Siberia (n=299)

Genotype	*Genotype frequency (%)*	*Allele frequency*	
Ser^{311}/Ser^{311}	95.98	Ser^{311} = 0.979	Cys^{311} =0.021
Ser^{311}/Cys^{311}	4.01		
Cys^{311}/Cys^{311}	-		

Table 2: Percentage distribution of DRD2 genotypes in different alcohol drinking groups

Genotype	*Alcohol consuming group*		
	Group 1	*Group 2*	*Group 3*
Ser^{311}/Ser^{311}	5.3%	84.0%	10.6%
Ser^{311}/Cys^{311}	-	91.7%	8.3%
Cys^{311}/Cys^{311}	-	-	-

Alcohol Drinking Habits vs. Genotypes

The majority of the subjects (84.7%) reported to consume alcohol only episodically (1-2 times a month). Only about 7% of the subjects reported to consume alcohol at least once per week (regular drinkers). The current and past abstainers constituted about 8.3%. The average dose of alcohol consumed per drinking occasion was about 27.2 g pure alcohol.

Alcohol-induced Flushing Response

Out of a total of 726 females questioned, 28.5% reported that they experienced facial flushing; 15.6% experienced flushing "sometimes" and 12.9% experienced flushing "often". The major-

ity of those females who experienced flushing (83.8%) were able to continue drinking after flushing began. However, 8.1% were not able to continue drinking and 8.1% could continue only sometimes. Many females reported to have adverse manifestations after alcohol ingestion (symptoms which usually accompany flushing). Nausea was reported by 0.3% of the often-flushed and by 2.3% of females who flushed sometimes only; in females reporting often and sometimes flushing, feeling drowsy was reported by 1.1% and 5.9%; feeling dizziness by 0.3% and 3.5%; increased heart beat by 0.6% and 2.2%; sweating by 0.3% and 1.8%, headache by 1.0% and 3.0%, respectively. Many of the flushers noted that not all types of drinks caused flushing but it was more often induced by vodka (42.6% cases) and sparkling wine (champagne) (32.6% cases).

No significant relationship between distribution of the Cys^{311} variants and alcohol drinking habits was noted. Moreover, no significant relationship between the genotype frequency and flushing response as well quantity of drinking and the prevalence of alcohol-related symptoms was observed. Taken together the present study do not hint to a strong association between Ser^{311}/Cys^{311} mutation and alcohol drinking habits in Siberian females. Moreover, as the mutant allele (Cys^{311}/Cys^{311}) is present in very low frequency in Siberians and other Europeans, it is difficult to assess its involvement in the etiology of alcohol abuse. Thus, the results of the present study support an earlier report (Goldman et al. 1998) showing that Ser^{311}/Cys^{311} mutation in DRD2 gene is not linked to alcohol abuse and /or alcoholism.

REFERENCES

Arinami T, Itokawa M, Enguchi H, Tagaya H, Yano S, Shimizu H, Hamaguchi H, Toru M 1994. Association of dopamine D2 receptor molecular variant with schizophrenia. *Lancet*, **343**: 703-704.

Avksentyuk AV, Muramatsu, Kurilovich SA, Voevoda MI 1995. Dopamnine D2 receptor Ser311/Cys311 alleles in Chukotka natives (Siberia). *Alcohol Alcoholism*, **30**: 553.

Belkovets A, Kurilovich S, Avkenstyuk A, Agarwal DP 2001. Alcohol drinking habits and genetic polymorphism of alcohol metabolism genes in West Siberia. *IJHG*, **1**: 151-156.

Blomqvist O, Gelernter J, Kranzler HR 2000. Family-based study of DRD2 alleles in alcohol and drug dependence. *Am J Med Genet*, **96**: 659-664.

Blum K, Noble EP, Sheridan PJ, Montgomery A, Ritchie T, Jagadeeswaran P, Nogami H, Briggs AH, Cohn JB 1990. Allelic association of human dopamine D2 receptor gene in alcoholism. *JAMA*, **263**: 2055-2060.

Blum K, Sheridan PJ, Wood RC, Braverman ER, Chen TJ, Comings DE 1995. Dopamine D2 receptor gene variants: association and linkage studies in impulsive-addictive-compulsive behaviour. *Pharmacogenetics*, **5**: 121-41.

Bobak M, McKee M, Rose R, Marmot M 1999. Alcohol consumption in a national sample of the Russian population. *Addiction*, **94**: 857-866.

Chen CH, Chien SH, Hwu HG 1996. No association of dopamine D2 receptor molecular variant Cys311 and schizophrenia in Chinese patients. *Am J Med Genet*, **67**: 418- 420.

Craddock N, Roberts Q, Williams N, McGuffin P, Owen MJ 1995. Association study of bipolar disorder using a functional polymorphism (Ser311—>Cys) in the dopamine D2 receptor gene. *Psychiatr Genet*, **5**: 63-65.

Crawford F, Hoyne J, Cai X, Osborne A, Poston D, Zaglul J, Dajani N, Walsh S, Bradley R, Solomon R, Mullan M 1996. Dopamine DRD2/Cys311 is not associated with chronic schizophrenia. *Am J Med Genet*, **67**: 483-484.

Finckh U, von Widdern O, Giraldo-Velasquez, Podschus J, Dufeu P, Sander T, Harms H, Schmidt LG, Rommelespacher H, Rolfs A 1996. No association of the structural dopamine D2 receptor (DRD2) variant ^{311}Cys with alcoholism. *Alcohol Clin Exp Res*, **20**: 528-532.

Finckh U 2001. The dopamine D2 receptor gene and alcoholism. Association studies. In: DP Agarwal, HK Seitz (Eds): *Alcohol in Health and Disease*. New York: Marcel Dekker, Inc. pp 151-176.

Galeeva AR, Iur'ev EB, Khusnutdinova EK 2000. Polymorphism of the dopamine D2 receptor gene in populations from the Volga-Ural region. *Genetika*, **36**: 1394-1400.

Gejman PV, Ram A, Gelernter J. 1994. No structural mutation in the dopamine D2 receptor gene. *J Neurochem*, **56**: 204-208.

Gelernter J, Kranzler H 1999. D2 dopamine receptor gene (DRD2) allele and haplotype frequencies in alcohol dependent and control subjects: no association with phenotype or severity of phenotype. *Neuropsychopharmacology*, **20**: 640-649.

Goldman D, Urbanek M, Guenther D, Robin R, Long JC 1998. A functionally deficient DRD2 variant [Ser311Cys] is not linked to alcoholism and substance abuse. *Alcohol*, **16**: 47-52.

Higuchi S, Muramatsu T, Murayama M, Hayashida M 1994. Association of structural polymorphism of the dopamine D2 receptor gene and alcoholism. *Biochem Biophys Res Commun*, **204**: 1199-1205.

Itokawa M, Arinami T, Futamura N, Hamaguchi H, Toru M 1993. A structural polymorphism of human dopamine D2 receptor (Ser311—>Cys311). *Biochem Biophys Res Commun*, **196**: 1369-1375.

Kaneshima M, Higa T, Nakamoto H, Nagamine M 1997. An association study between the Cys311 variant of dopamine D2 receptor gene and schizophrenia in the Okinawan population. *Psychiatry Clin Neurosci*, **51**: 379-381.

Kidd KK, Pakstis AJ, Castiglione CM, Kidd JR, Speed WC, Goldman D, Knowler WC, Lu RB, Bonne-Tamir B 1996. DRD2 haplotypes containing the TaqI A1 allele: implications for alcoholism research. *Alcohol Clin Exp Res*, **20**: 697-705.

Kidd KK, Morar B, Castiglione CM, Zhao H, Pakstis AJ, Speed WC, Bonne-Tamir B, Lu RB, Goldman D,

Lee C, Nam YS, Grandy DK, Jenkins T, Kidd JR 1998. A global survey of haplotype frequencies and linkage disequilibrium at the DRD2 locus. *Hum Genet*, **103**: 211-227.

Kurilovich SA, Jakuschenko IA, Egorova NG, Avksentyuk AV, Trusov VB 1998. Flushing response and its role in alcohol disease in Siberian populations. *Int J Circumpolar Health*, **57 Suppl 1:** 454-458.

Lawford BR, Young RM, Rowell JA, Gibson JN, Feeney GF, Ritchie TL, Syndulko K, Noble EP 1997. Association of the D2 dopamine receptor A1 allele with alcoholism: medical severity of alcoholism and type of controls. *Biol Psychiatry*, **41**: 386-393.

Lu RB, Lee JF, Ko HC, Lin WW 2001. Dopamine D2 receptor gene (DRD2) is associated with alcoholism with conduct disorder. *Alcohol Clin Exp Res*, **25**: 177-184.

Nacher V 2000. Genetic association between the reduced amplitude of the P300 and the allele A1 of the gene which codifies the D2 dopamine receptor (DRD2) as possible biological markers for alcoholism. *Rev Neurol*, **30**: 756-763.

Nanko S, Hattori M, Dai XY, Fukuda R, Kazamatsuri H 1994. DRD2 Ser3l1/Cys3l1 polymorphism in schizophrenia. *Lancet*, **343**: 1044.

Nemtsov AV 2000. Estimates of total alcohol consumption in Russia, 1980-1994. *Drug Alcohol Depend*, **58**: 133-142.

Noble EP 2000. The DRD2 gene in psychiatric and neurological disorders and its phenotypes. *Pharmacogenomics*, **1**: 309-333.

Parsian A, Cloninger CR, Zhang ZH 2000. Functional variant in the DRD2 receptor promoter region and subtypes of alcoholism. *Am J Med Genet*, **96**: 407-411.

Pato CN, Maccardi F, Pato MT, Verga M, Kennedy JL 1993. Review of the putative association of dopamine D2 receptor and alcoholism. A meta-analysis. *Am J Med Genet*, **48**: 78-82.

Sander T, Harms H, Podschus J, Finckh U, Nickel B, Rolfs A, Rommelspacher H, Schmidt LG 1995. Dopamine D1, D2 and D3 receptor genes in alcohol dependence. *Psychiatr Genet*, **5**: 171-176

Serretti A, Macciardi F, Smeraldi E 1998. Dopamine receptor D2 Ser/Cys311 variant associated with disorganized symptomatology of schizophrenia. *Schizophr Res*, **34**: 207-210.

Sobell J, Sigurdson DC, Heston L, Sommer S 1994. S311C D2DR variant: no association with schizophrenia. *Lancet*, **344**: 621-622.

Spurlock G, Williams J, McGuffin P, Aschauer HN, Lenzinger E, Fuchs K, Sieghart WC, Meszaros K, Fathi N, Laurent C, Mallet J, Macciardi F, Pedrini S, Gill M, Hawi Z, Gibson S, Jazin EE, Yang HT, Adolfsson R, Pato CN, Dourado AM, Owen MJ 1998. European Multicentre Association Study of Schizophrenia: a study of the DRD2 Ser311Cys and DRD3 Ser9Gly polymorphisms. *Am J Med Genet*, **81**: 24-28.

Tanaka T, Igarashi S, Onodera O, Tanaka H, Fukushima N, Takahashi M, Kameda K, Tsuji S, Ihda S 1996. Lack of association between dopamine D2 receptor gene Cys311 variant and schizophrenia. *Am J Med Genet*, **67**: 208-211.

Verga M, Macciardi F, Pedrini S, Cohen S, Smeraldi E 1997. No association of the Ser/Cys311 DRD2 molecular variant with schizophrenia using a classical case control study and the haplotype relative risk. *Schizophr Res*, **25**: 117-121.