

알코올의존 환자의 도파민 수송체(DAT1)G2319A의 유전자 다형성 연합연구*

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Association Study of Dopamine Transporter(DAT1) G2319A Genetic Polymorphism in Alcohol Dependence*

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ABSTRACT

Objective : Dopamine transporter is member of family of Na/Cl dependent neurotransmitter transporter, 12 transmembrane domain, that has high substrate specificity, affinity. It is related with dopamine reuptake in presynaptic vesicle. DAT has a VNTR in its 3' - untranslated region(UTR). 3' - UTR VNTR polymorphism is related with modification of dopamine transmission.

The association between with VNTR polymorphism and neuropsychiatric disorders such as alcohol dependence, and low activity ALDH has been studied, but their relationship is unclear. We study about association of 3' - UTR VNTR of DAT gene and G2319A and alcohol dependence.

Method : Group of Korean subjects were studied with alcohol dependence(n=49 male) compared to mentally healthy controls(n=53 male). The peripheral blood sample was acquired, and Polymerase Chain Reaction(PCR) amplification, MspI procedure was done.

Result : There was a significant difference between alcohol dependence group and normal control(genotype frequency $p < 0.05$, allele frequency $p < 0.05$) Allele A frequency and genotype(GG, GA) frequency was a significant difference between alcohol dependence group and normal control($p < 0.05$).

Conclusion : Our study showed that genetic polymorphism of DAT1 G2319A had relation with alcohol dependence.

KEY WORDS : Alcohol dependence · Genetic polymorphism · Dopamine transporter.

서 론

(Cloninger 1991).

, 가

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가 (cocaine)

가 (amphetamine)

(receptor), (transporter) 가 (reverse transport) (Jones 1998). MPTP(1 - methyl - 4 - phenyl - 1, 2, 3, 6 - tetrahydropyridine)

alcohol dehydrogenase(ADH) 가 (Gainet-dinov 1997).

aldehyde dehydrogenase(ALDH), ALDH2^{2/2}가 (transmembrane protein) , 12 가 , sodium chloride

(1999 ; Agarwal 1987 ; Crabb 1993 ; Goedde 1987 ; Sherman 1995). 66% 가

(coding) Tiihonen (1995) (striata) (dopamine transporter densities) SPECT 가

가 (rewarding system) (circuit) , Tupala (2000) (,)

(ventral tegmental area) (nucleus accumbens) 가 Cloninger(1987)

(euphorogenic effect) , Kuikka (1998a) Volkow (1996)

(reward effect) , Tupala (2000)

가 cDNA cDNA

가 Vandenberg (1992) , (DAT1 : SLC6A3) 5p15.3

cleft 가 3가 (polymorphism) , highly polymorphic intron 8 TaqI Variable Number of Tandem Repeat(VNTR)(Byerley 1993), biallelic TaqI Restriction Fragment Length Polymorphism(RFLP)(“ TaqI 492 ”)(Vandenberg 1992b), 3 ' (untranslated region : UTR) 40bp VNTR(Sano 1993 ; Vandenberg 1992a)가 . 40 bp VNTR in the 3 ' - UTR 가 64kb

A1 가 15 exon spanning (Donovan (1997 ; Noble 1991 ; Parsian 1991). 1995 ; Kawarai 1997). VNTR (repeat range)가 3 11 copy , 가 10copy가 (Doucette - Stamm (Ritz 1987), 1995 ; Nakatome 1995 ; Nakatome 1996 ; Persico

1996), 9copy (Gelernter 1998 ; Kang 1999). 40bp VNTR(7 copy)가 가 가 1 ALDH ALDH2*2/2* (Mura- matsu 1995) , A9 copy가 가 (Sander 1997).

Gelernter (1994) DAT1 (3'-VNTR 9 re- peated allele) cocaine induced paranoia가 , Persico (1995) (complete linkage)

(ADHD)

ADHD 가 . DAT1 VNTR, 40bp VNTR ADHD 가 가 Cook (1994) . Curran (2001) 480bp VNTR

가

ALD ALDH 가 , 가 . 3'-UTR VNTR G2319A . G2319A DAT1 3'-UTR genomic DNA cDNA , DAT1 cDNA 2319 G A , MspI 가 . MspI ,

실험재료 및 방법

1. 연구 대상

1998 DSM- (American Psychiatric As- sociation 1994) 49 ,

CAGE(Cut down, Annoyed, Guilty, Eye open) 가 1 AUDIT(Alcohol Use Disorder Identi- fication Test) 가 9 53 . 18 65

2. 혈액채취 및 DNA분리

DNA 5ml heparine - lithium tube - 20 . DNA , 0.1N NaOH 0.2ml 3 , 0.4ml , phenol extraction (phenol/chloroform 1 : 1) 2 , 1/10 sodium acetate(3M, pH 5.2) , 1/2 ice - cold absolute alcohol 가 - 70 10 DNA . Eppendorf 14,000rpm 10 , ice - cold 70% al- cohol 가 , salt . 10 14, 000rpm DNA pallet 10ul DNA . genomic DNA 100ng/ul (Polymerase Chain Reaction : PCR)

3. Genomic DNA 증폭-PCR

DNA PCR RFLP (1998). primer GenBank accession number M95167(Homo sapiens dopamine transporter(SLC6A3)) , primer Primer Detective program(ClonTech) sense primer 5'-CCGTGTCTTG-TGTTGCTGTA-3' anti-sense primer 5'-ACGGGGATTCTCAGCAG-GTG-3' . PCR (1998) . , DNA 0.1ug/ul (10mM Tris - HCL, pH 9.0, 50mM KCl, 0.1% Triton X - 100) 60ul 10mM dNTPs(Beorin- ger Maanheim), 2U Taq DNA polymerase, 0.5uM primer , 94 30 , 57 30 , 72 30 35 cycle 72 5 .

4. MspI digestion

PCR 217bp DNA fragment , PCR Msp 137bp 80bp fragment . PCR 40ul 1U Msp 37 . 20% acrylamide gel ,

3 . ethidium bromide ($\chi^2=6.737, df=1, p=0.009$)
 , 2 가 가 , GA ($\chi^2=5.400, df=1, p=0.020$)가 . AA sample 가
 (variant type) A 가 A
 217bp 가 A
 AA , 137bp 80bp 가 GG , ($\chi^2=7.118, df=1, p=0.004$)가
 217bp, 137bp 80bp 가 GA G
 ($\chi^2=1.441, df=1, p=0.230$)가 .

5. 전기영동-Acrylamide gel running

Msp digestion product 8% polyacrylamide gel
 110V 1 running

고 찰

6. 통계분석

chi square Fisher's exact test
 , SPSS/PC+ version 7.5 . χ^2 -test, Fisher's
 extract, probability test
 $p < 0.05$

가 , GG
 GA
 G 가 ,
 A 가 .
 A
 A가
 (phenotype) 가 .
 가 3'-UTR
 3'-UTR 가
 mRNA
 (localization), (transcript stability),
 Goodwin
 (1993) 3'-UTR 가 (regulatory
 protein) 가 , 3'-UTR
 (polymorphism) 9 copy 가 10 copy
 가 22%
 (Heinz 2000).
 Grunhage (2000) (regulatory reg-
 ion) (linkage disequilibrium) 가 가

결 과

49 53
 1
 49 GG 27 (55%), GA
 21 (43%), AA 1 (2%) , G가
 77%, A가 23% . 53 GG
 11 (21%), GA 39 (73%), AA 3 (6%) ,
 G 58%, A 42% .
 ($\chi^2=12.999, df=1, p=0.003$)
 가 , ($\chi^2=$
 8.258, $df=2, p=0.004$) 가 .
 , GG

Table 1. Genotype and allele frequencies of G2319A polymorphism of the human DAT1 gene in alcoholics and in controls

	Genetic frequency(n/%) [†]			Allele frequency(n/%) [‡]			
	Total	GG	GA	AA	Total	G*	A**
Alcoholics	49	27 (55%)	21 (43%)	1 (2%)	98	75 (77%)	23 (23%)
Control	53	11 (21%)	39 (73%)	3 (6%)	106	61 (58%)	45 (42%)
Total	102	38	60	4	204	136	68

n : number of subject
 allele G means the wild-type allele, and allele A, the variant in G2319A polymorphism
 Chi-square test
[†] : χ^2 : 12.999 p : 0.003, [‡] : χ^2 : 8.258 p : 0.004
 * : χ^2 : 1.441 p : 0.230, ** : χ^2 : 7.118 p : 0.004
 : χ^2 : 6.737 p : 0.009, : χ^2 : 5.400 p : 0.020

가 .
 3'-UTR 40bp VNTR
 copy 가 . A9 copy A10 copy
 가 가 ,
 가
 (Sander 1997). , 40bp VNTR
 ,
 (Cook
 1994 ; Curran 2000 ; Rowe 1998).

Ueno (1999) G2319A 가 (Sander 1997).
non - A10 , A10 , 가 A10 SEPCT PET Cloninger
가 (Martinez 2001). , Martinez 가 , 가 .
(temperament)
(temperament - character inventory : TCI) 4가
A (allele) A9 A10 가
가 GA , 가 (Sabol 1999). (2000)
A 가 (Monoamine oxidase)ACA
G A가
가 가 , 가 , 가
가 가 , A (match) 가
가 가 , Ueno (1999) (,)
G2319A G 가 . Ueno (selection bias)
(1999) copy
가 Kang (1999) copy (Ueno 1999)
가 Kang (1999)
SLC6A3 copy , ,
10 copy 9 copy가 가
copy , copy 가
가 (subtype) 가
), , 가 (,
(temperament) 가 , 가
A9 ,

중심 단어 :

참고문헌

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