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Association between the polymorphism in the promoter region of dopamine D4 receptor gene and chronic tic disorder

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Abstract: **Objective** To study a possible association between the three functional polymorphisms in the promoter region of dopamine D4 receptor (DRD4) gene and chronic tic disorder. **Methods** Genomic DNA was isolated from the venous blood leukocytes of 84 unrelated patients with chronic tic disorder (Study group) and 100 healthy unrelated individuals (Control group). Polymorphisms of DRD4, -1240L/S, -616C/G and -521C/T, were genotyped by the allele-specific primer (ASP) PCR. Genotype, allele and haplotype frequencies were analysed by SHEsis online. **Results** There were significant differences in both allele and genotype frequencies ($\chi^2 = 8.419$, $P < 0.01$; $\chi^2 = 7.860$, $P < 0.05$ respectively) of DRD4-616C/G between the Study and the Control groups. Haplotypic frequencies of LCT (-1240L/S, -616C/G, -521C/T) in the Study group were noticeably higher than in the Control group ($\chi^2 = 6.371$, $P < 0.05$). **Conclusions** There is an association between the DRD4-616C/G polymorphism and chronic tic disorder. The individuals with haplotype LCT (-1240L/S, -616C/G, -521C/T) are susceptible to this disorder.

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Key words: Chronic tic disorder; Dopamine D4 receptor; Polymerase chain reaction; Allele specific amplification; Haplotype

多巴胺 D4 受体基因启动子区多态性与慢性抽动障碍的关联研究

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[**摘　要**] **目的**　探讨多巴胺 D4 受体(DRD4)基因启动子区的 3 个功能多态性与慢性抽动障碍是否存在相关性。**方法**　选取无亲缘关系的慢性抽动障碍患儿 84 例以及无亲缘关系的健康个体 100 例,后者作为对照组。提取静脉血白细胞基因组 DNA,采用聚合酶链反应及等位基因特异性扩增技术检测 DRD4 基因启动子区 -1240L/S,-616C/G和-521C/T 3 个功能位点的基因型。用 SHEsis 在线统计软件分析各位点等位基因、基因型、单倍型频率及其组间差异。**结果**　DRD4 基因 -616C/G 的等位基因频率及其基因型频率在慢性抽动障碍组显著高于正常对照组($\chi^2 = 8.419$, $P < 0.01$; $\chi^2 = 7.860$, $P < 0.05$), DRD4 基因-1240L/S, -616C/G 和-521C/T 组成的单倍型 LCT 的频率在慢性抽动障碍组显著高于正常对照组($\chi^2 = 6.371$, $P < 0.05$)。**结论**　DRD4 基因 -616C/G 的等位基因可能与慢性抽动障碍相关联,携带有 DRD4 基因-1240L/S, -616C/G 和-521C/T 组成的单倍型 LCT 的个体可能更易患慢性抽动障碍。

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[**关　键　词**]　慢性抽动障碍;多巴胺 D4 受体;聚合酶链反应;等位基因特异性扩增; 单倍型

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Chronic tic disorder is a childhood/adolescent-onset neuropsychiatric disorder, characterized by multiple motor and vocal tics. Genetic studies in twins and families provide compelling evidence that tic disorder is a disease of polygenic inheritance [1,2]. Dopamine receptors are important compositions of dopaminergic system and in recent years, an association of dopamine D4 receptor (DRD4) and some neuropsychiatric disorders

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has been reported [3,4]. Many studies on the association between DRD4 gene and tic disorder have focused on the variation of the 48-bp variable number of tandem repeat (VNTR) in the third exon, however, the results were not consistent. Recent sequence analysis at the promoter region of the DRD4 gene has identified several new polymorphisms in this region, many of which are believed to affect transcription of the gene [5-8]. In this paper, three polymorphisms in the promoter region (-1240L/S, -616C/G, -521C/T) were tested using a case-control design. The aim of this study was to elucidate more precisely the region of the gene, and to investigate whether any polymorphisms are associated with chronic tic disorder by specific alleles and multi-marker haplotypes .

Materials and methods

Subjects

A total of 84 cases of unrelated chronic tic disorder were Han Chinese in origin (63 males and 21 females), aged between 7-18 years, with a mean age of 14.2 ± 3.8 years. All cases were recruited from the Department of Developmental Pediatrics, Second Affiliated Hospital, China Medical University. All patients were definitely diagnosed with chronic tic disorder based on the diagnostic criteria of Diagnostic & Statistical Manual for Mental Disorders (DSM Ⅳ, USA). These patients were used as the Study group. One hundred unrelated healthy control subjects were Han Chinese in origin (56 males and 44 females), with a mean age of 15.5 ± 2.5 years.

Methods

Genomic DNA was extracted from venous blood using a KI method [9]. Polymerase chain reaction (PCR) cycling was performed on the DNA thermal cycler (Gene Amp 9700, Perkin-Elmer, USA). The genotyping of the -1240L/S polymorphism was performed according to Seaman *et al* [10] and PCR products were run out on 2% agarose gel stained with GeneFinder. The PCR reaction yielded distinct bands at 429 bp was designated as short allele (S, without tandem duplication) and that at 549 bp was designated as long allele (L, with tandem duplication). A 100 bp ladder determined the size of products. The genotyping of the -616C/G and -521C/T SNPs was performed by single-tube allele-specific PCR (SAS-PCR) according to Ronai *et al* [11] and Ronai *et al* [12], respectively. See Table 1.

Table 1 Primers and condition of PCR

Polymorphism	Primer sequence	Annealing temp(℃)	Product
-1240L/S	F: GTTGTCTGTCTTTTCTCATTGTTTCCATTG	65	L:549bp
	R: GAAGGAGCAGGCACCGTGAGC		S:429bp
-616C/G	F: GAACCTACCCCGGCCTGTCGT	69	645bp
	R: AGACGGGAATGAAGCGAGGTGG		
	C specific: TGGTCGCGGGGGCTGAGC		415bp
	G specific: CCCCCCMGCAGCCTCTGGYC		267bp
-521C/T	F: GGAATGGAGGAGGGAGCGGG	69	605bp
	R: CGCTCCACCGTGAGCCCAGTAT		
	C specific: GGAGCGGGCGTGGAGGGC		405bp
	T specific: GCCTCGACCTCGTGCGCA		235bp

Statistical analysis

Comparisons of the allele, genotype and haplotype frequencies between the Study and the Control groups were performed using SHEsis online program (http://nhgg.org/analysis/) [13], and the χ^2 value, the odds ratio (*OR*) and their 95% confidence intervals (*CI*) were calculated. A value of $P < 0.05$ was considered statistically significant.

Results

Genotype and allele frequencies of three polymorphisms in the promoter region of DRD4

The distribution of three polymorphisms (-1240L/S, -616C/G, -521C/T) of the DRD4 gene was in Hardy-Weinberg equilibrium. The data for genotype and allele frequencies of the three polymorphisms are shown in Table 2. C allele frequencies of -616C/G of the DRD4 gene in the Study group were significantly higher than in the Control group ($\chi^2 = 8.419$, $P = 0.004$, $OR = 2.04$, 95% $CI = 1.25 - 3.32$). There were also significant differences in the genotype frequencies of -616C/G between the two groups ($P = 0.020$, $\chi^2 = 7.860$). No significant differences in the allele and genotype frequencies of -1240L/S and -521C/T were found between the two groups.

Table 2 Frequencies of genotype and allele of the three polymorphisms

Position	*n*	Genotype count (frequency)			Number of allele (frequency)	
-1240L/S		LL	LS	SS	L	S
Control group	100	35(0.35)	50(0.5)	15(0.15)	120(0.6)	80(0.4)
Study group	84	38(0.452)	35(0.417)	11(0.131)	111(0.661)	57(0.339)
-616C/G		CC	CG	GG	C	G
Control group	100	4(0.04)	28(0.280)	68(0.68)	36(0.18)	164(0.82)
Study group	84	9(0.107)[a]	34(0.405)[a]	41(0.488)[a]	52(0.310)[b]	116(0.690)
-521C/T		CC	CT	TT	C	T
Control group	100	12(0.12)	54(0.54)	34(0.34)	78(0.39)	122(0.61)
Study group	84	14(0.167)	44(0.524)	26(0.310)	72(0.429)	96(0.571)

a vs the Control group, $P<0.05$ (comparison of genotype frequency of -616C/G); b vs the Control group, $P<0.01$ (comparison of C allele frequency of -616C/G).

Haplotype frequencies of three polymorphisms in the promoter region of DRD4

The haplotype frequencies of LCT (-1240L/S, -616C/G, -521C/T) in the Study group were noticeably higher than in the Control group ($\chi^2=6.371$, $P=0.012$, $OR=2.984$, 95 % $CI=1.233$ -7.223). See Table 3.

Table 3 Haplotype frequencies of the three polymorphisms

Group	Haplotype (%)							
	LCC	LCT	LGC	LGT	SCC	SCT	SGC	SGT
Control	4.6	3.7	13.1	38.6	4.9	4.8	16.4	13.9
Study	8.2	10.4	17.1	30.5	7.8	4.6	9.7	11.7
χ^2 value	2.007	6.371	1.156	2.668	1.346	0.009	3.512	0.370
P value	0.157	0.012	0.282	0.102	0.246	0.926	0.061	0.543
OR	1.850	2.984	1.370	0.697	1.649	0.955	0.550	0.826
95% *CI*	0.781-4.379	1.233-7.223	0.771-2.434	0.451-1.076	0.703-3.865	0.360-2.531	0.292-1.034	0.446-1.531

Discussion

Chronic tic disorder is a complex disease. Current evidence suggests that tic disorder may result from a defect in the dopaminergic system. Dopamine receptors are important compositions of dopaminergic system. A great deal of attention has been focused on the possible involvement of DRD4 following a report of an association of tic disorder with a 48-bp VNTR polymorphism in the third exon, but there have been several non-replications of this association. Grice *et al* [14] reported an association between the 7-repeat allele of DRD4 and tic disorder. It is important to note, however, that there have been several non-replications of this association [15-17]. The failure of several studies to replicate the association may result from population ethnicity [18].

In this study three polymorphisms in the promoter region (-1240L/S, -616C/G, -521C/T) were tested. There were significant differences in C allele frequencies of DRD4-616C/G between the patients with chronic tic disorder and healthy controls, suggesting that there is an association between the DRD4-616C/G polymorphism and chronic tic disorder. Barr [19] searched the sequence of -616C/G for potential DNA transcription factor binding sites. The C to G sequence change at -616 potentially resulted in a gain of an AP-2 binding site. AP-2 is an inducible and developmentally regulated family of transcription factors. The C to G sequence change at -616 potentially affected the activity of AP-2 binding site, thereby restraining the induction and transcription of DRD4. It was speculated that C allele of -616C/G may reduce AP-2 binding site, and restrain transcription of DRD4.

Haplotypic frequencies of LCT (-1240L/S, -616C/G, -521C/T) in patients with chronic tic disorder were noticeably higher than in the controls, which suggested that there is an association between the haplotype LCT and chronic tic disorder. The longer allele has lower transcription activity than the shorter allele in the -1240L/S polymorphism [5]. The C to T change at -521, has been reported to change the levels of transcription by 40% [6]. In this study, a significant increase in C allele of -616C/G in patients with chronic tic disorder was observed when compared with the controls. It was speculated that C allele of -616C/G may reduce AP-2 binding site, and restrain transcription of DRD4. Haplotype LCT composed of the three polymor-

phisms in the promoter region of DRD4 (-1240L/S, -616C/G, -521C/T) may restrain transcription activity of DRD4, and decrease DRD4 gene expression and increase susceptibility to chronic tic disorder.

In conclusion, the results of the current investigation show an association between polymorphisms in the promoter region of DRD4 and chronic tic disorder. The individuals with C allele of -616C/G may be susceptible to chronic tic disorder. The individuals with haplotype LCT (-1240L/S, -616C/G, -521C/T) may be more susceptible to chronic tic disorder. In the future, a study on gene polymorphisms and gene expression is warranted to perform by *in vivo* or *vitro* trials to show how the gene variant decides the expression and induction of DRD4, in order to elucidate the pathogenesis of chronic tic disorder and offer a basis for diagnosis and treatment of this disorder.

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