行政院國家科學委員會專題研究計畫 成果報告

妥瑞症基因之相關性研究

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(一)中文摘要

研究背景與目的：妥瑞症是兒科重要的常見神經精神疾病。多項證據指向妥瑞症是源自發展中的腦基底核，出現多巴胺的高反映性。多巴胺一號受體基因是神經與精神疾病的重要標的基因，與多巴胺代謝有關。根據我們實驗室過去的研究基礎，我們利用單核酸多形性(single nucleotide polymorphisms; SNPs)為工具，以之 SNP 為標記，尋找妥瑞症與多巴胺一號受體基因之關聯，以明白妥瑞症與多巴胺之機轉。

實驗方法： 以中國醫藥大學附設醫院小兒神經科,就診的妥瑞症的臺灣病童為實驗對象。對照組收集亦在同家醫院,同為臺灣人種族,家族不相關聯,且無妥瑞症之家族史者。總數為 148 名病童與 83 比名對照組。多形性基因為多巴胺一號受體基因 DRD1,以 polymerase chain reaction 的方式分析。兩組的基因型分布與對偶質的頻率相比。

結果: 我們發現多巴胺一號受體基因 DRD1 中在實驗組與對照組的差異並無統計上的意義。

結論: 多巴胺一號受體基因 DRD1 與妥瑞症並無相關聯。這可以提供吾人對妥瑞症的認識，更可提供將來對妥瑞症的研究方向。

關鍵詞：妥瑞症、單核酸多形性、多巴胺、多巴胺一號受體基因

(二)英文摘要

Objective: Recent research suggests that Tourette's syndrome(TS) may result from a defect in the dopamine system. The dopamine 1 receptor (DRD1) gene is a candidate gene in the study of the etiology of neuropsychiatric diseases that may involve dopaminergic abnormalities. We sought to test the hypothesis that the DRD1 gene might play a role in TS.

Methods: By performing an association study, we collected an independent sample of patients from the midland region of Taiwan and investigated whether DRD1 gene polymorphisms can be used as markers of susceptibility to TS. A total of 148 children with TS and 83 normal control subjects were included in the study. A polymerase chain reaction was used to identify the A/G polymorphism of the DRD1 gene. Genotypes and allelic frequencies for the DRD1 gene polymorphisms in both groups were compared.

Results: The results showed that genotypes and allelic frequencies for the DRD1 gene polymorphisms in both groups were not significantly different.

Conclusion: These data suggest that DRD1 gene may not be a useful marker for prediction of the susceptibility of TS.

Keywords: Tourette's syndrome, dopamine D1 receptor gene, DRD1, polymorphism

(三)報告內容

Introduction

Gilles de la Tourette syndrome (TS) is a neuropsychiatric disorder characterized by both motor and vocal tics. In addition, affected individuals frequently display symptoms such as attention deficit hyperactivity disorder and or obsessive–compulsive disorder. In the 1970s, investigators first demonstrated that TS shows a familial concentration (Eldridge et al., 1977). To date, the gene search in TS has been unsuccessful (Heutink et al., 1993) and is illustrative of the many factors that can complicate genetic analysis of complex human traits (Comings, 1995). Current evidence suggests that TS may result from a defect in the dopamine system (reviewed in Leckman et al., 1988). This hypothesis is supported by the successful reduction of tics in the majority of patients using neuroleptics (dopaminergic blocking agents) (Shapiro et al., 1989). Tic suppression also has been reported with an agent that blocks the accumulation of dopamine in presynaptic storage vesicles, tetrabenazine (Jankovic et al., 1989), and an agent that blocks dopamine synthesis, alpha methylparatyrosine (Sweet et al., 1974). Many reports have emphasized the interaction between dopamine 1 and dopamine 2 receptors (DRD1 and DRD2) in a wide range of behaviors including schizophrenia, cataplexy, substance abuse, and other behaviors. In contrast to the extensive studies of the DRD2 gene, there have been few studies of the DRD1 gene in TS (Comings et al., 1997; Thompson et al., 1998). The dopamine D1 receptor's role in many brain functions (O'Dowd, 1993) and its high affinity for dopamine (Sunahara et al., 1990) indicated the importance of DRD1 gene integrity to dopamine pathways. These observations lead us to test the polygenic hypothesis by examining the potential effect of DRD1 in TS. In this study, we tested the hypothesis that genetic variation in the DRD1 gene confers susceptibility to TS. Two single nucleotide polymorphism (SNP) markers have been identified in SNP155417 (Ser421Ser), at nucleotide position 1263, and the G-A nucleotide exchange in the 50 untranslated region (50-UTR), respectively, allowing researchers to detect disease causing gene associations (http://www.ncbi.nlm.nih.gov/SNP).

Patients and methods

The study included Taiwanese children with Tourette's syndrome (group 1, n=148) and normal control subjects (group 2, n=83). This study was approved by the Ethics Committee of the China Medical University Hospital, Taichung, Taiwan. All parents signed informed consent before blood tests were performed. Cases were matched with controls according to age, sex, ethnicity and geographic location of origin. TS subjects and the controls were both recruited from the midland regions of Taiwan. Diagnosis of TS followed the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSMIV). The criteria for TS are as follows: the presence of multiple motor tics and at least one vocal tic (not necessarily concurrently); a waxing and waning course with tics evolving in a progressive manner; the presence of tic symptoms for at least 1 year; the onset of symptoms before age 18; the absence of a precipitation illness or medication; and marked distress or significant impairment in social, occupational, or other important areas of functioning. All cases were from unrelated kindred. The 83 controls were healthy volunteers with no history of tics and other psychiatric disorder. All the children accepted the peripheral blood sampling for genotype analyses. Genomic DNA was isolated from peripheral blood using the DNA extractor Genomaker DNA extraction kit (Blossom). The primer sequences for fragment amplification and polymerase chain reaction conditions were carried out as described elsewhere (Comings et al., 1997). The polymorphisms were analyzed by polymerase chain reaction amplification followed by restriction analysis: PvuI for DRD1 (SNP155417), and DdeI for DRD1 (50-UTR). Genotypes and allelic frequencies for DRD1 (SNP155417) and DRD1 (50-UTR) polymorphisms in both groups were compared. The SAS system with the chi-squared test was used for statistical analyses. $P<0.05$ was considered statistically significant.

Results

No one carries the SNP155417 polymorphism in the DRD1 gene in either group, so there were no data or statistical analysis. Genotype proportions for DRD1 (50-UTR) were significantly different, but allele frequencies were not significantly different in both groups (Table 1).

Discussion

In the present study, we found that children with TS and normal controls lacked expression of polymorphisms in DRD1 (SNP155417). This discrepancy with previous reports from Western countries may have been due to racial variation (http://www.ncbi.nlm.nih.gov/SNP). Furthermore, we found that TS patients are not associated with DRD1 (50-UTR) gene polymorphisms. This finding is consistent with recent studies demonstrated that DRD1 does not contribute to the etiology of TS (Comings et al., 1997; Thompson et al., 1998). Thus, although sharing important clinical features with other neuropsychiatric diseases, TS may not share a genetic etiology of mutations in DRD1 gene.

The role of dopaminergic system in the pathogenesis of TS is still unknown. Preliminary studies have suggested that the pathogenesis of tics involves neuronal activity within subcortical neuronal circuits (Peterson et al., 1998). Therefore, the classic neurotransmitters, dopamine and serotonin, raised the possibility that they may be involved in the pathobiology of TS. However, other investigators have emphasized that abnormalities of dopamine fail to explain many clinical and laboratory observations, including the description of unchanged tics in four adults who developed parkinsonism and received treatment with L-dopamine (Kumar and Lang, 1997). Besides, limited studies of D1 and D2 receptor binding in postmortem striatal tissue show trends but no significant differences between TS and control members (Singer et al., 1991).

TS of children may involve a complex interaction between the environmental influences, especially infection, autoimmune contributions, epigenetic factors and genetic factors. Our study suggests that the DRD1 gene may not contribute to the etiology of TS. Further studies could be focused on the analysis of other dopaminergic genes in TS patients. Our results could provide the database for further survey of the DRD1 polymorphisms.

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Table 1. Genotypes for DRD1 (5'-UTR) polymorphisms in children with Tourette's syndrome and normal controls

	Tourette's No.(%) (n = 148)	Normal controls No.(%) (n =83)	P-value*
Genotype			
AA	119(80.4)	60(72.3)	0.016
AG	23(15.5)	23(27.7)	
GG	6(4.1)	0	
Allelic frequency			
A	261(88.2)	143(86.1)	0.627
G	35(11.8)	23(13.9)	

* p-value were calculated by χ^2 test

(四)計畫成果自評部份

妥瑞症是兒科重要的常見神經精神疾病。因其研究只是近二十多年的事，其發生率常被低估，從每萬人口約有 1 到 10 例，到 1996 年發生率高達 1/200 以上的報告出爐。因其症狀跨越神經與精神科學領域，故成為研究人類神經精神異常的“ 典範 ”。妥瑞症的遺傳特性是不爭的事實，但卻不是單純的顯性、隱性或性聯遺傳方式可以解釋，其遺傳方式應非單一基因，而是多基因遺傳。遺傳標記現仍尋找中。

單核酸多形性(single nucleotide polymorphisms; SNPs)是人體基因體中最常見的基因差異，因其具有快速，便宜及精確的特性，故以 SNP 為標記可以作為尋找與疾病相關聯基因的最佳工具。本實驗以 SNPs 為研究模式去探討以下四大類的基因是否與妥瑞症有關聯，目前初步的發現多巴胺受體基因 DRD1 gene 並無相關聯，此研究成果已被 SCI 際期刊 Psychiatric Genetics 接受 (見附錄，PDF 檔)。未來將再就其它多巴胺基因作更進一步的確定。此外，因妥瑞症的合併症過動與注意力不集中症候群，與正腎上腺素分泌過多有關，將研究第二種類的正腎上腺素基因。第三種是血清素，因妥瑞症的病人在中樞神經與血小板中的血清素分泌偏低。與第四種是神經傳導物質 γ-胺基丁酸，因它是抑制性的神經傳導物質，而妥瑞症是抑制神經失調，再深入與基因標記對照。這不僅可以提供吾人對妥瑞症成因的認識，研究其致病機轉，更可提供將來對其他神經精神疾病新的治療與預防方向。