A Family-Based Association Study of Attention-Deficit Hyperactivity Disorder and Dopamine D2 Receptor TaqI A Alleles

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Background: Over the past 5 years, considerable progress has been made in the identification of polymorphic variation within monoamine system genes that are associated with the attention-deficit hyperactivity disorder (ADHD) phenotype. In this study, we investigated the association of the dopamine D2 receptor (DAD2) TaqI A and ADHD in a Taiwanese sample.

Methods: The sample consisted of 98 children with ADHD and 154 of their parents. ADHD cases were ascertained from the Child Psychiatric Clinics at Chang Gung Memorial Hospital in the Taipei area, Taiwan. A diagnosis of ADHD was made following clinical interviews plus completion of a standard maternal interview and Conner's revised rating scales by a parent and teacher. Association of DRD2 TaqI A polymorphism in this sample was investigated using a haplotype-based haplotype relative-risk method.

Results: Among our subjects, there was no significant difference in transmission rates between DRD2 TaqI A1 and A2 alleles.

Conclusion: The results of this study do not support DRD2 playing a major role in Taiwanese children with ADHD.
(Chang Gung Med J 2003;26:897-903)

Key words: attention-deficit hyperactivity disorder, dopamine D2 receptor, TaqI A, association study.

Attention-deficit hyperactivity disorder (ADHD) is a clinical disorder characterized by a persistent pattern of overactivity, inattention, and impulsivity that is severe, developmentally inappropriate, and accompanied by impaired function at home and school. ADHD is also one of the most common disorders in child clinical populations. Mannuzza et al.[1] reported that children with ADHD are at heightened risk for lower educational attainment, lower income, and underemployment. Moreover, they suffer higher risks of dropping out of school, adult criminality, and substance abuse.[1-3] Longitudinal studies indicate that over 1/3 of children with ADHD go on to have significant behavioral and psychiatric problems in adulthood. ADHD is associated with a variety of adverse environments, many of these factors involving parent-child relationships.[4]

Despite the seriousness of ADHD, little is known about its causes. In recent years, it has become apparent that genetic influences are an important part of the etiology of ADHD.[5] Family[6,7] and adoption[8] studies have suggested that ADHD is

familial, and that a genetic influence may contribute to its etiology. Data on twin are used to estimate heritability, which measures the degree to which a disorder is influenced by genetic factors. By analyzing twin data, Gillis et al.[9] concluded that ADHD is highly heritable.

The effectiveness of stimulants, along with animal models of hyperactivity, point to catecholamine dysregulation as at least 1 source of ADHD brain dysfunction.[10] Several molecular genetic studies of ADHD have focused on the genes that are involved in dopaminergic function, because of the central role of dopamine in motor activity and reward-seeking behaviors. For the known dopaminergic markers that are currently reported to be associated with ADHD, odds ratios have been estimated at 1.4 and 1.16 for the dopamine D4 receptor gene (DRD4)[11] and dopamine transporter gene (DAT1)[12], respectively, on the basis of meta-analyses of available data.

Cook et al.[13] revealed an association between ADHD and the 480-bp allele of DAT1 using a family-based association study. This finding was replicated by Waldman et al.[5] and Gill et al.[14]. The association of DAT1 and ADHD is of particular interest, given that the psychostimulant medications that are the most frequent treatments of choice for ADHD exert their pharmacological effects in part by inhibiting the dopamine transporter and thus keeping a greater quantity of dopamine active in the synaptic cleft for a longer period of time.[15] To date, there have been 10 published association studies of ADHD with a 480-bp allele of a variable-number tandem repeat polymorphism in the 3'-untranslated region of the gene, 6 support an association with the 10-repeat allele and 4 do not.[12] Recently we analyzed DAT1 polymorphisms in a Taiwanese sample of ADHD proband-parent trios.[16] Interestingly, we found considerable evidence for linkage and association with excess transmission of the 10-repeat allele from heterozygous parents.

The human dopamine D2 receptor (DRD2) gene is located on chromosome 11q22-23, and consists of 8 exons separated by 7 introns.[17] The DRD2 is a binding site of many psychoactive drugs, and it has been proposed to be a genetic risk factor for psychiatric disorders. Several polymorphisms were identified shortly after the gene was cloned, 3 of which are TaqI restriction fragment length polymorphisms.

The first, termed TaqI A, has been the most commonly studied in association with psychiatric diseases. There is also considerable variation in the distribution of TaqI A alleles in different ethnic and racial populations.[18]

Comings[19] suggested that the A1 allele of the DRD2 gene is associated with a number of behavioral disorders in which it may act as a modifying gene rather than as the primary etiological agent. The DRD2 gene appears to be one of these, since variants at this locus are significantly increased in frequency in Tourette syndrome, ADHD, conduct disorder, and drug abuse.[20] Rowe et al.[21] reported that heritability from the DRD2 locus was estimated to be 4.27% for hyperactive-impulsive symptoms and 2.12% for inattentive symptoms, and that children with the A2A2 genotype had the highest mean level of symptoms. However, results of a study by Todd et al.[22] did not support the DRD2 gene contributing to susceptibility for ADHD. In this study, we attempted to investigate the association of the DRD2 gene with ADHD in Taiwanese children in a family-based sample.

METHODS

In total, 98 subjects (83 males and 15 females) were recruited from children being assessed and/or treated for ADHD at the Department of Child Psychiatry, Chang Gung Children's Hospital, Taipei. Written informed consent was obtained from their parents after introduction to this study. All subjects were assessed with (1) the Conners Child Activity Rating Scale completed by a caretaker and teacher; (2) a clinical diagnosis by a board-certificated child psychiatrist; (3) and a clinical interview or structured interview with the Schedule for Affective Disorder and Schizophrenia for School-Age Children, Adolescent Version (K-SADS-E) by an experienced child psychiatrist. The collected information was incorporated into the operational instrument, "Hypescheme International".[23]

Buccal cells were collected from subjects and their parents by the cheek swab method. DNA extraction was performed using commercial kits. Restriction fragment length polymorphism analysis of TaqI A polymorphism of the DRD2 gene was carried out using a polymerase chain reaction (PCR)-based restriction analysis according to the method

described by Grandy et al.[24] In brief, the sequences of sense and antisense primers were 5'-CCG TCG ACG GCT GGC CAA GTT GCT CTA-3' and 5'-CCG TCG ACC CTT CCT GAG TGT CAT CA-3' respectively. The 30-μl volume PCR mixture contained 1X PCR buffer, 1.5 mM MgCl$_2$, 0.25 mM dNTPs, 1 μM of each sense and antisense primer, 1 unit of DNA Tag polymerase, and 50 ng of genomic DNA. After initial denaturation at 94°C for 5 min, 30 cycles of the PCR reaction were performed under conditions of denaturation at 95 °C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 1 min, with a final extension of 10 min at 72°C. Ten microliters of the PCR products was then digested with 5 units of TaqI restriction enzyme in buffer with a total volume of 20 μl at 65°C for at least 8 hours. Digested PCR products were separated by electrophoresis in a 2% agarose gel, and visualized with ethidium bromide staining under ultraviolet light. The A1 allele was characterized by a band of 310 bp, whereas for the A2 allele, this band was digested into 2 bands of 180 and 130 bp.

Analysis of allele associations was performed using a haplotype-based haplotype relative-risk method (HHRR).[25] By reference to the proband's genotype, each parental allele was scored as to whether it was or was not transmitted. The total collections of transmitted and non-transmitted alleles were considered as 2 independent case-control samples. We also analyzed the association after stratifying the probands into sporadic cases and familial cases, i.e., those with at least 1 sibling with ADHD.

RESULTS

The sample consisted of 98 children with ADHD and 154 of their parents. The age of the subjects ranged from 5 to 15 years, with a mean of 8.8 (SD=2.5) years. Among the subject, 83 (84.7%) were male and 16 (15.3%) were female. The mean gestational age of these probands when they were born was 38.3 (SD=1.5) weeks. The details are shown in Table 1. Among these 98 probands, 16 had siblings with ADHD, and were grouped as familial cases.

Information on the transmission of alleles was obtained from 56 trios and 42 parent/proband pairs. Twelve of the parent/proband pairs were doubly heterozygous and thus informative. As shown in Table 2, the transmission rate was 53.3% for the A1 allele

and 49.7% for the A2 allele. There was no significant difference in the transmission rates between these 2 alleles. There was no significant association between Taq I A polymorphism and ADHD when the probands were stratified into male and female groups, or into sporadic cases and familial cases.

Table 1. Demographics of the Probands with Attention-Deficit Hyperactivity Disorder

	Sporadic cases N = 82	Familial cases N = 16	Total N = 98
Age (years)	8.6±2.3	9.5±3.0	8.8±2.5
Gestational age (weeks)	38.3±1.6	38.3±1.0	38.3±1.5
≤ 38	34 (41.5)	5 (31.2)	39 (39.8)
> 38	37 (45.1)	9 (56.3)	46 (46.9)
Missing	11 (13.4)	2 (12.5)	13 (13.3)
Gender			
Male	73 (89.0)	10 (62.5)	83 (84.7)
Female	9 (11.0)	10 (37.5)	16 (15.3)

Percentages are given in parentheses.

Table 2. Haplotype Relative-Risk Analysis

		Transmitted	Not transmitted	X^2	p
Total	A1	56 (53.3)	49 (46.7)	0.3	0.557
	A2	89 (49.7)	90 (50.3)		
Sporadic cases	A1	47 (51.6)	44 (48.4)	0.04	0.842
	A2	77 (50.3)	76 (49.7)		
Familial cases	A1	9 (64.3)	5 (35.7)	1.2	0.273
	A2	12 (46.2)	14 (53.8)		
Male	A1	47 (53.4)	41 (46.6)	0.4	0.544
	A2	76 (49.4)	78 (50.6)		
Female	A1	9 (52.9)	8 (47.1)	0.004	0.952
	A2	13 (52.0)	12 (48.0)		

Percentages are given in parentheses.

DISCUSSION

Among our subjects, there was no significant association between Taq I A polymorphism and ADHD. Sixteen probands among our subjects (16.3%) had at least 1 sibling with a diagnosis of ADHD. Our subjects were not randomly sampled, and this rate cannot represent the actual concurrent rate among siblings in the ADHD population. Previous reports[6,7] demonstrated that familial or genetic factors greatly contribute to the etiology of ADHD.

The strategies for exploring the location of susceptible genes that are available for polygenetic disorders are genetic linkage and association studies. Linkage studies look for evidence for co-segregation between diseases and genetic markers in families with multiple affected individuals.[26] However, since most psychiatric disorders may reflect the operation of many genes each with small effects, the linkage approach requires very large samples of families to have adequate power to detect the genes.[26,27] Fortunately the sample sizes required for association studies are feasible even if the disease allele contributes only a small effect size.[26,28,29] Association studies attempt to determine whether a genetic variant is more common among affected than among non-affected individuals, but they have their own limitations. For example, they are prone to false positives due largely to population stratification and multiple testing.[29] Family-based association methods can avoid the effects of population stratification.[30] Therefore, in this study, we attempted to obtain DNA samples from probands and their parents. The common statistical methods for association analysis using cases and parental controls are the haplotype relative risk (HRR)[31] and the transmission disequilibrium test (TDT)[32]. The sampling unit of the HRR is a family with 2 parents and a single affected offspring. Each unit contributes a case genotype (the genotype of the affected offspring) and an artificially constructed 'control genotype' made up of 2 remaining alleles of the 2 parents not transmitted to the affected offspring. The original HRR considers the case genotypes and the control genotypes as   2 independent samples, and uses a standard methodology for unmatched case-control studies. The method we used in this study is a popular variation of the HRR, called haplotype-based HRR (HHRR), which considers the allele rather than the genotype as the unit of observation. The HRR or HHRR ignores the fact that transmitted and non-transmitted alleles contributed by a parent can be regarded as paired observations, and may be misleading under the situation of non-random mating. The TDT, a McNemar test for matched-pair data, considers only parents whose transmitted and non-transmitted alleles differ, and assesses the evidence for preferential transmission of 1 allele over the other. In some circumstances, the TDT may lose some of the information available in the sample.

The negative results observed in this study should not necessarily be considered a failure to replicate previous studies that reported significant associations of the DRD2 with ADHD. The term 'failure to replicate' should be reserved for studies of the same population using similar ascertainment and diagnostic practices. Discrepant results may be real and reflect heterogeneity with a genetic risk factor playing a quantitatively or qualitatively different role in different diagnostic or ethnic groups.[29] For conducting association studies in psychiatric genetics, Owen et al.[29] made some recommendations. First and most importantly, they suggested studying samples of adequate size. With a sample composed of 56 trios and 30 informative parent/proband pairs, this study might not have had sufficient power to detect small to moderate effects of the markers we studied for ADHD. Nevertheless, the results of this study suggest that DRD2 plays no major role in Taiwanese children with ADHD.

Although Rowe et al.[21] estimated that the heritability from the DRD2 locus was 4.27% for hyper-active-impulsive symptoms and 2.12% for inattentive symptoms, their results were non-significant in tests that controlled for population heterogeneity.[21] Using groups of individuals who met the diagnostic criteria for DSM-IV-defined ADHD subtypes, as well as recently defined latent class criteria for pure familial forms of ADHD, Todd et al.[22] reported that no coding region sequence variations in the DRD2 gene were identified to contribute to susceptibility for ADHD.

Morrison and Stewart[33] and Faraone and Biederman[10] suggested that ADHD may be caused by several interacting genes with modest effects. Like many physical diseases and virtually all psychiatric disorders, ADHD can be considered a complex trait from a genetic perspective.[34] Given its non-Mendelian transmission pattern, the lack of a simple 1-to-1 genotype-phenotype relationship, reduced penetration of any putative liability-increasing alleles, and the presence of phenocopies, ADHD must be approached by using contemporary molecular genetic analytic methods.[5] New technologies that allow the rapid screening in association studies may be applied to these samples in the future.

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注意力缺失過動症與多巴胺D2受器TaqI A基因之
家族樣本關聯分析

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背　景： 過去五年，在尋找單胺系統中與過動症有關聯之基因變型，已有相當的進步。本研究針對華人家族樣本進行過動症與多巴胺D2受器 (DRD2) TaqI A基因之關聯分析。

方　法： 本研究收集長庚醫院北部院區兒童心智門診就診之過動症病患98名及病患雙親154名，過動症病患乃經由兒童精神科醫師以臨床會談，及家長與老師填寫的 "Conner's 兒童活動量表" 記錄於診斷整合工具 "Hypescheme International" 後達成診斷，並以 haplotype-based haplotype relative risk方法進行統計分析DRD2 TaqI A基因變型與過動症之關聯。

結　果： 在此樣本中，DRD2 TaqI A1與A2 allele之傳遞率未達統計上有意義之差異。

結　論： 本研究的結果並不支持DRD2 TaqI A在過動症華人兒童中扮有重要角色。
(長庚醫誌 2003;26:897-903)

關鍵字： 注意力缺失過動症，多巴胺D2受器基因，TaqI A，關聯分析。