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Dopamine Receptor D4 (*DRD4*) Gene in Han Chinese Children With Attention-Deficit/Hyperactivity Disorder (ADHD): Increased Prevalence of the 2-Repeat Allele

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There is an increased prevalence of the 7-repeat (7R) allele of the dopamine receptor D4 (*DRD4*) gene in attention-deficit/hyperactivity disorder (ADHD). However, the population prevalence of the 7R allele varies considerably across ethnicity and is very low in Asians. To test whether this 7R allele/ADHD association still held in a Chinese clinical sample, 32 Han Chinese children with a confirmed ADHD diagnosis and normal IQ who were methylphenidate-responders were genotyped. None of them had a *DRD4* 7R allele. Instead, we observed a significantly increased prevalence of the 2-repeat (2R) allele in this clinical sample (33%) compared to ethnically-matched controls (20%) (χ^2(1d.f.) = 5.90, $P = 0.015$). This approximately 1.65-fold increase of the 2R allele in our probands is close to the observed increase of the 7R allele in European-ancestry ADHD children. Recent genetic studies have indicated that the 2R allele in Asians is likely derived from the 7R allele. Further, available biochemical data indicate that both the 2R and 7R protein have blunted responses to dopamine compared to the 4R protein. Based on these results, we propose that the observed increased prevalence of the 2R allele in our Han Chinese ADHD probands is still consistent with the 7R allele hypothesis of ADHD in European-ancestry children. Recent studies have suggested that any variant from the conserved ancestral 4R allele might potentially alter biochemistry/phenotype. We hypothesize that an increased frequency of any non-4R allele may define the association of the *DRD4* gene with ADHD that holds across ethnicity. The present findings, however, obtained with a small ADHD sample size, should be replicated.

KEY WORDS: ***DRD4*; ADHD; Han Chinese; 2-repeat allele; 7-repeat allele**

A positive association had been found between attention-deficit/hyperactivity disorder (ADHD) and the increased prevalence of the 7-repeat (7R) allele of a 48 bp variable number of tandem repeats (VNTR) in the exon III of the dopamine receptor D4 (*DRD4*) gene located on chromosome 11p15.5 [LaHoste et al., 1996; Swanson et al., 1998a; see Swanson et al., 2000; and Faraone et al., 2001 for reviews]. Pharmacological, neurochemical, and neuroanatomical studies of ADHD provided plausible hypotheses about this association based upon possible functions of the *DRD4* gene that might be altered by the 7R allele [Asghari et al., 1995; Barr, 2001]. In populations of European ancestry, 43%–49% of ADHD probands had at least one 7R allele compared to 21%–23% in controls [LaHoste et al., 1996; Rowe et al., 1998; Swanson et al., 1998a; Grady et al., 2003], due to the increased prevalence of the 7R allele (22%–27% vs. 12%–13%, respectively) [Rowe et al., 1998; Holmes et al., 2000; Mill et al., 2001; Grady et al., 2003].

The prevalence of the 7R allele varies considerably across ethnicity and is very low in Asians, notably Han Chinese [Chang et al., 1996; Ding et al., 2000, 2002]. However, the prevalence of ADHD in Han Chinese children is similar to that observed in European-ancestry children [Swanson et al., 1998b]. Han Chinese ADHD children exhibit the same behavioral symptomatology, cognitive deficits, and developmental delays as their European-ancestry counterparts [Ho et al., 1996; Leung et al., 1996]. Given the now well-replicated association between the 7R allele and ADHD, the former could still be present at increased frequency in Han Chinese ADHD children, despite its low prevalence (0%–2%) in the general population [Chang et al., 1996; Ding et al., 2000, 2002]. Alternatively, the *DRD4* 7R allele might have little association with ADHD in Han Chinese, and this finding should prompt efforts to examine other alleles/genes.

Thirty-seven Han Chinese children with a clinical diagnosis of ADHD were recruited from two child psychiatric clinics in

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Hong Kong. A favorable clinical response to methylphenidate for at least a period of 3 months was an additional inclusion criterion because some previous studies had found the association between the 7R allele and ADHD in samples that excluded non-responders to methylphenidate [e.g., LaHoste et al., 1996; Swanson et al., 1998a; Tahir et al., 2000]. Exclusion criteria for participation were diagnoses of mental retardation, autism, and physical disabilities. The study protocol and consent form were approved by the respective ethics committees of the universities and hospitals involved, complying with the Codes of Ethics of the World Medical Association (Declaration of Helsinki). Informed written consent for participation was provided by the parents for their children. Ten c.c. of venuous blood were taken from these 37 children for genotying. Blood samples or DNA were sent to the Department of Biochemistry, University of California, Irvine, and genotyping was conducted as described previously [Ding et al., 2002; Grady et al., 2003]. The Vocabulary subtest of the HK-WISC (Hong Kong Wechsler Intelligence Scale for Children) was administered to these children to estimate a Verbal IQ.

The parents of these children were interviewed by the Diagnostic Interview Schedule for Children—Version IV (DISC-IV) which yield Diagnostic and Statistical Manual of Mental Disorders—4th Edition (DSM-IV) diagnoses for children and adolescents [Shaffer et al., 2000]. In this study, an English to Chinese translation of the Parent version was used and four modules on anxiety disorders, mood disorders, disruptive behavior disorders, and alcohol/substance abuses were administered. The DISC-IV diagnosis of ADHD was used to confirm the clinical diagnosis of the local clinicians in Hong Kong and to ensure standardization of case definition and compatibility with other studies of ADHD.

The clinical ADHD diagnosis of five children was not confirmed by the DISC-IV. Given their favorable responses to medication, their condition in the last year might have been improved to a stage that it no longer met the full diagnostic criteria of ADHD and became subclinical. Consequently, the group of DISC-confirmed ADHD probands consisted of 32 Han Chinese boys whose age ranged from 6 to 15 years (mean = 9.1, SD = 1.9) and whose verbal IQs from 75 to 145 (mean = 109, SD = 18). Fourteen of them (44%) met the criteria for the combined type, 10 (31%) for the inattentive type, and 8 (25%) for the hyperactive-impulsive type. Twenty-five children (78%) also had at least one or more comorbid DISC-IV diagnoses (mean = 1.6, SD = 1.4), which is consistent with the baseline assessment in the Multimodality Treatment Study of ADHD [MTA Cooperative Group, 1999]. Comorbid externalizing disorders were present in 23 cases (22 with oppositional defiant disorder and 1 with conduct disorder), and comorbid internalizing disorders were present in 29 cases, including 14 cases of specific phobia, 6 cases of social phobia, 2 cases of separation anxiety disorder, 2 cases of generalized anxiety disorder, 2 cases of obsessive-compulsive disorder, 1 case of agoraphobia, and 2 cases of dysthymic disorder.

Genotying revealed that none of our 32 probands had a 7R allele (Table I). The same result held for the five cases that met our clinical but not DISC-IV criteria for ADHD. These data do not support a priori hypothesis of an increased presence of the 7R allele in Han Chinese ADHD children. Interestingly, the 2-repeat (2R) allele was found to be increased instead (Tables I and II). Compared to 247 *DRD4* genotypes from five Han Chinese community samples surveyed previously (San Francisco (N = 49), Taiwan (N = 43), Liaoning (N = 67), Qingdao (N = 35), and Guangdong (N = 43)) [Chang et al., 1996; Ding et al., 2002], we observed over twice the number of ADHD children with a 2R/X genotype in our clinical sample than expected by chance (Table I). Table II presents the result of a Chi-square analysis, confirming a significant increase in the frequency of the 2R allele in our Han Chinese ADHD probands (33%) compared to ethnically-matched controls from the five Han Chinese communities mentioned above (20%) (χ^2(1d.f.) = 5.90, $P = 0.015$). If we included our five removed cases into the analysis, the result was almost identical, i.e., 32% versus 20%, χ^2(1d.f.) = 6.29, $P = 0.012$. This approximately 1.65-fold increase of the 2R allele in our ADHD probands is similar to the increase of the 7R allele in European-ancestry ADHD children compared to controls [LaHoste et al., 1996; Rowe et al., 1998; Swanson et al., 1998a; Holmes et al., 2000; Mill et al., 2001; Grady et al., 2003].

Unlike some previous studies [e.g., LaHoste et al., 1996; Swanson et al., 1998a], we included in our sample not only ADHD (combined type), but also the two other types (inattentive or hyperactive-impulsive type). Although the combined type reported a somewhat higher frequency of the 2R allele (36%) than the other two types (31%), the difference was not significant at these small sample sizes (χ^2(1d.f.) = 0.19, $P = 0.66$).

Population stratification is a concern in candidate gene studies, but the prevalence of the 2R allele among our five control samples was uniform (18% in San Francisco, 20% in Taiwan, 22% in Liaoning, 16% in Qingdao, and 22% in Guangdong). So the average 20% prevalence of the 2R allele in our composite control group is representative of the ethnicity of our clinical sample (Han Chinese). Furthermore, in 10 non-Han Chinese ethnic minorities surveyed previously (2N = 458, Lisu, Uyger, Yi, Tibetan, Buyi, Dai, Hani, Lu, Pumi, and Wa nationalities), all had *DRD4* 2R allele frequencies similar to or lower than those of the Han Chinese, except for the Wa in Yunnan Province [Ding et al., 2000]. Thus, it is unlikely that population stratification by itself can adequately explain the observed increased prevalence of the 2R allele in our Han Chinese ADHD probands.

Recent studies have examined the sequences of individual motifs of *DRD4* exon III alleles and their linkage disequilibrium (LD) with adjacent polymorphisms [Ding et al., 2002; Wang et al., 2004]. Ding et al. [2002] reported strong LD between an adjacent A-C SNP pair and the 7R allele. However, in the Asian subsample of this study, all 26 Asian 2R alleles

TABLE I. Observed and Expected Frequencies of the *DRD4* Genotypes of 32 Han Chinese ADHD Probands

	Genotype						
	2R/2R	2R/3R	2R/4R	2R/5R	3R/4R	4R/4R	4R/5R
Observed	0	1	19	1	1	10	0
Expected	1	<1	9	<1	1	18	1

Computation of the frequencies of expected genotypes was based upon the *DRD4* allele frequencies of five community samples of Han Chinese (2N = 494) and Hardy–Weinberg equilibrium was assumed (2R = 0.196, 3R = 0.018, 4R = 0.745, 5R = 0.022, 6R = 0.008, 7R = 0.010, 8R and greater <0.001). Genotypes which are not found to occur in our clinical sample and which would have been expected to occur <1 time given the allele frequency of the community samples are not included in this table. For the five ADHD children excluded by DISC evaluation, the observed genotypes were 2R/4R (two cases), 4R/4R (two cases), and 2R/6R (one case).

TABLE II. Comparison of *DRD4* Allele Frequencies (2R Vs. Non-2R Alleles) Between 32 Han Chinese ADHD Probands and 247 Ethnically-Matched Community Controls

	Allele			
	2R	Non-2R		Freq (2R)
Controls	97	397	494	0.20
Probands	21	43	64	0.33
	118	440	558	

$\chi^2(1 \text{ d.f.}) = 5.90$, $P = 0.015$.

examined were linked to the A-C SNP pair, suggesting that the 2R allele in Asians originated from recombinations involving the 7R allele. Further resequencing analysis has shown unequivocally that the 2R allele is a recombination product of the 7R allele [Wang et al., 2004]. Biochemical analysis has demonstrated that the 7R protein, compared to the 4R protein, has a three-fold blunted ability to reduce cAMP levels [Asghari et al., 1995]. In this same study, the 2R protein also exhibited a blunted cAMP response, although midway between those of the 4R and 7R variants. Based on these genetic and biochemical findings, the absence of the 7R allele in our Han Chinese probands does not necessarily reject the 7R allele hypothesis of ADHD. The haplotype of the 2R allele in our probands is likely derived from the 7R allele and functions to some extent similarly as the latter [Wang et al., 2004]. This possibility revives a variant of the 7R allele hypothesis of ADHD in Han Chinese.

The common 4R (1-2-3-4) haplotype has been identified as the conserved ancestral allele [Ding et al., 2002; Wang et al., 2004]. Any variant from it might potentially alter biochemistry/phenotype [Grady et al., 2003; Wang et al., 2004]. It was hypothesized that ADHD, instead of a specific association with the 7R allele, might be associated with any allelic variant (e.g., 2R/X genotype in Han Chinese or 7R/X genotype in populations of European ancestry) that differed from the ancestral 4R/4R genotype.

This study demonstrates the value of cross-ethnic research. Prior studies with European-ancestry ADHD children resulted in a focus on the 7R allele or allele length. Our findings here from a different ethnic group, Han Chinese, suggest a different focus. Studies by Ding et al. [2002], Grady et al. [2003], and Wang et al. [2004] imply that the evolutionary history of the *DRD4* alleles, whether referring to the ancestral relationship between the 2R and 7R alleles in Asians, or to allelic variants evolving from the ancestral 4R allele, may be relevant to understand why the 2R and 7R alleles are associated with ADHD in different ethnic groups. *DRD4* 2R and 7R alleles share a recent evolutionary history [Wang et al., 2004] which may account for the common clinical consequences of these variations despite the apparent diversity of their prevalence across ethnicity.

We recognize the limitations of our case-control study with a small ADHD sample and the risk of population stratification. Despite these limitations, this research serves well as a pilot study to propose new hypotheses and directions, moving beyond focusing on the 7R allele of the *DRD4* gene and on the ADHD probands of European ancestry.

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