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

Patrick W.L. Leung,1,4 C.C. Lee,2 S.F. Hung,2 T.P. Ho,3 C.P. Tang,2 S.L. Kwong,2 S.Y. Leung,4 S.T. Yuen,3 F. Lieh-Mak,5 Jaap Oosterlaan,6 Deborah Grady,7 Ante Harxhi,7 Y.C. Ding,7
H.C. Chi,7 Pamela Flodman,8 Sabrina Schuck,8 M. Anne Spence,8 Robert Moyzis,7,8 and James Swanson8

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%) ($\chi^2$(1.d.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.

*Correspondence to: Patrick W.L. Leung, Department of Psychology, The Chinese University of Hong Kong, Hong Kong

H.C. Chi, Pamela Flodman, Sabrina Schuck, M. Anne Spence, Robert Moyzis, and James Swanson

Department of Pediatrics, University of California, Irvine, Irvine, California

Department of Biological Chemistry, University of California, Irvine, California

Department of Pathology, University of Hong Kong, Hong Kong

Department of Psychiatry, University of Hong Kong, Hong Kong

Department of Psychiatry, Queen Mary Hospital, Hospital Authority, Hong Kong

Department of Psychology, The Chinese University of Hong Kong, Hong Kong

Department of Psychology, The Chinese University of Hong Kong (Mainline Research Grant); Grant sponsor: University of California, Irvine (Pacific Rim Grant); Grant sponsor: National Institute of Health; Grant number: MH60660.

© 2005 Wiley-Liss, Inc.
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 venous blood were taken from these 37 children for genotyping. 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 confirmed by the DISC-IV. Given their favorable responses to medication, their condition in the last year might have been confirmed by the DISC-IV. Given their favorable responses to medication, their condition in the last year might have been confirmed by the DISC-IV.

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>2R/2R</th>
<th>2R/3R</th>
<th>2R/4R</th>
<th>2R/5R</th>
<th>3R/4R</th>
<th>4R/4R</th>
<th>4R/5R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>0</td>
<td>1</td>
<td>19</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Expected</td>
<td>1</td>
<td>&lt;1</td>
<td>9</td>
<td>&lt;1</td>
<td>1</td>
<td>18</td>
<td>1</td>
</tr>
</tbody>
</table>

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).
examined were linked to the A-C SNP pair, suggesting that the 2R allele in Asians originated from recombination involving the 7R allele. Further resequencing analysis has shown unequivocally that the 2R allele is a recombinant 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.

REFERENCES


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

<table>
<thead>
<tr>
<th>Allele</th>
<th>Controls</th>
<th>Proband</th>
<th>Freq (2R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2R</td>
<td>97</td>
<td>97</td>
<td>0.20</td>
</tr>
<tr>
<td>Non-2R</td>
<td>397</td>
<td>21</td>
<td>0.33</td>
</tr>
<tr>
<td>494</td>
<td>0.20</td>
<td>21</td>
<td>0.33</td>
</tr>
<tr>
<td>0.20</td>
<td>397</td>
<td>21</td>
<td>0.33</td>
</tr>
</tbody>
</table>

χ²(1 d.f.) = 5.90, P = 0.015.