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# Genes and attention-deficit hyperactivity disorder

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**Abstract**

In a collaborative research program on attention-deficit hyperactivity disorder (ADHD) initiated 20 years ago at UC Irvine, we adopted Cantwell's (1994) approach to define a refined phenotype for use in studies of the biological bases of this disorder. We have used this refined phenotype (ADHD-Combined Type without internalizing comorbidities) in our molecular genetic studies of ADHD, which have paralleled the emerging literature in this new field. In our research program, we used the candidate gene approach, with hypotheses derived from the dopamine theory of ADHD and Posner and Raichle's (1994) theory of attention. We proposed a candidate dopamine gene (DRD4) and discovered an association with ADHD due to an increase prevalence of the '7-repeat' allele defined by a 48-base-pair variable number of tandem repeats in exon III. The DRD4–ADHD association has now been confirmed by multiple groups around the world. In the next steps of our research program, we are evaluating the impact of a putative DRD4 risk allele on cognition, initiating an investigation of DNA sequence variation in DRD4 alleles, and investigating the association of ADHD with other candidate genes. Using our collaborative research program as an example, we will review the history and current status of molecular genetic studies of ADHD. 

*Keywords*: Attention-deficit hyperactivity disorder; Refined phenotype; Dopamine gene DRD4

## 1. A refined phenotype of ADHD

Attention-deficit hyperactivity disorder (ADHD) is considered to be the most prevalent psychiatric disorder of childhood. For over a half century the clinical definition has been refined and a specific pharmacological intervention (low doses of stimulant drugs such as amphetamine or methylphenidate) has been confirmed as an effective treatment for this condition (see Ref. [1]). Despite a long history, widespread clinical acceptance, and extensive research, the diagnosis of ADHD and its treatment with stimulant medication is still controversial. In fact, at a recent NIMH Consensus Conference [2], some vocal critics even denied that this condition should be called a disorder, citing the lack of clear evidence of a biological etiology.

For over 20 years, we have engaged in a collaborative research program at the University of California, Irvine (UCI) to evaluate possible biological bases of ADHD. Starting in the 1980s this program focused on the validity of the diagnosis [3] and the genetics [4] of ADHD, in collaboration with Dennis Cantwell at UCLA. In the early 1990s, the focus shifted to possible neuropsychological and neuroanatomical bases [5], in collaboration with Michael Posner at the University of Oregon [6]. In the mid-1990s the focus narrowed to address molecular genetics, initially in collaboration with James Kennedy and Cathy Barr at the University of Toronto [7,8] and later with Robert Moyzis and Anne Spence at UCI [9]. Recently we [10] summarized the initial phases and here we place emphasis on the later phases of this collaborative research program.

The same 18 symptoms of ADHD are listed in the Diagnostic and Statistical Manual, Version IV (DSM-IV [11]) published by the American Psychiatric Association and in the International Classification of Diseases, Edition 10 (ICD-10 [12]) manual published by the World Health Organization as criteria for hyperkinetic disorder (HKD). These behaviors can be defined as psychopathology that is present or absent (see Table 1) or as the extremes of underlying dimensions that encompass normal behavior (see Table 2). In our research program, we have used rating scales to collect norms for these behaviors in school

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Table 1
DSM-IV/ICD-10 symptoms: the SNAP rating scale with items defined by psychopathology

| | For each item, check the column that best describes this child: | Not at all | Just a little | Quite a bit | Very much |
|---|---|---|---|---|---|
| 1. | Often fails to give close attention to detail or makes careless mistakes in schoolwork or tasks | _____ | _____ | _____ | _____ |
| 2. | Often has difficulty sustaining attention in tasks or play activities | _____ | _____ | _____ | _____ |
| 3. | Often does not seem to listen when spoken to directly | _____ | _____ | _____ | _____ |
| 4. | Often does not follow through on instructions and fails to finish school work, chores, or duties | _____ | _____ | _____ | _____ |
| 5. | Often has difficulty organizing tasks and activities | _____ | _____ | _____ | _____ |
| 6. | Often avoids, dislikes, or reluctantly engages in tasks requiring sustained mental effort | _____ | _____ | _____ | _____ |
| 7. | Often loses things necessary for activities (e.g. toys, school assignments, pencils, or books) | _____ | _____ | _____ | _____ |
| 8. | Often is distracted by extraneous stimuli | _____ | _____ | _____ | _____ |
| 9. | Often is forgetful in daily activities | _____ | _____ | _____ | _____ |
| 10. | Often fidgets with hands or feet or squirms in seat | _____ | _____ | _____ | _____ |
| 11. | Often leaves seat in classroom or in other situations in which remaining seated is expected | _____ | _____ | _____ | _____ |
| 12. | Often runs or climbs excessively in situations in which it is inappropriate | _____ | _____ | _____ | _____ |
| 13. | Often has difficulty playing or engaging in leisure activities quietly | _____ | _____ | _____ | _____ |
| 14. | Often is 'on the go' or often acts as if 'driven by a motor' | _____ | _____ | _____ | _____ |
| 15. | Often talks excessively | _____ | _____ | _____ | _____ |
| 16. | Often blurts out answers before questions have been completed | _____ | _____ | _____ | _____ |
| 17. | Often has difficulty awaiting turn | _____ | _____ | _____ | _____ |
| 18. | Often interrupts or intrudes on others (e.g. butts into conversations or games) | _____ | _____ | _____ | _____ |

wide samples, using the Swanson, Nolan and Pelham (SNAP) rating scale of psychopathology and the Strengths and Weaknesses of ADHD-symptoms and Normal-behavior (SWAN) rating scale of underlying dimensions of attention and action. These norms can be used to quantify statistical abnormality based on severity as judged by parents and teachers (see Fig. 1). Some complexities of setting cutoffs for severity are discussed in detail elsewhere [13], but percentiles (for the SNAP) or standard scores (for the SWAN) can be used to specify a cutoff to identify a specific percentage of school-aged children in the top end of the distribution (e.g. 5%).

It is notable that in earlier versions of the DSM and ICD manuals, the specific symptoms listed were different for ADHD and HKD, so this convergence at the symptom level represents progress toward a unified definition of the disorder [1]. However, the decision rules for implementing the DSM-IV and ICD-10 criteria still differ in terms of inclusion criteria (DSM-IV allows the diagnosis of partial syndromes of ADHD-Inattentive and Hyperactive-Impulsive subtypes but ICD-10 does not) and exclusion criteria (DSM-IV recommends the diagnosis of ADHD in the presence of non-externalizing comorbid disorders but ICD-10 does not).

Differences in severity and decision rules can result in major differences in the phenotype defined, and this has been cited as a primary reason for large differences in the estimated prevalence of ADHD and HKD in school-aged children – from almost 25% for the least restrictive methods for the diagnosis of ADHD to about 1% for the most restrictive methods for diagnosis of HKD [1]. However, when similar operational definitions are applied in epidemiological studies, about the same estimates of prevalence of ADHD/HKD are obtained around the world for any given definition.

In prior publications [1,14] we proposed a refined phenotype of ADHD/HKD, defined by the full syndrome without serious comorbid conditions, for use in initial investigations of biological bases of this disorder. This corresponds to the overlap of the ADHD and HKD criteria [1,14]. In clinical samples, not all cases with a diagnosis of ADHD will meet the criteria for this refined phenotype. For example, in a large trial of a new medication [15], 27% of the cases met the DSM-IV criteria for a partial syndrome (20% with ADHD-Inattentive Type and 7% with ADHD-Hyperactive/Impulsive Type), and thus would fail to meet the criteria for the refined phenotype of ADHD/HKD. In a large trial of multimodality treatment of ADHD (MTA [16]), 41% of the cases with ADHD-Combined Type had a comorbid disorder other than Oppositional Deficit Disorder (ODD) or Conduct Disorder (CD), which would meet the exclusion criteria for the refined ADHD/HKD phenotype.

If these exclusion criteria are considered and accepted, then one would expect that less than 50% ($0.73 \times 0.59 = 0.43$) of the typical clinical cases in the United States might meet the restrictive criteria for refined ADHD/HKD phenotype. Epidemiological studies (see Ref. [1]) suggest that the prevalence of this restrictive phenotype is between 1 and 3% of the school-aged population. Since most of our studies of the biological bases of ADHD used this phenotype, the conclusions drawn from our research program may not hold for the broader, less restrictive phenotype used in clinical practice (see Refs. [1,6–10,13,14]).

Table 2
DSM-IV/ICD-10 symptoms reworded: the SWAN rating scale with items defined by dimensions encompassing normal behavior

| | Compared to other children, how does this child do the following: | Far below avg. | Below avg. | Slightly below avg. | Avg. | Slightly above avg. | Above avg. | Far above avg. |
|---|---|---|---|---|---|---|---|---|
| 1. | Give close attention to detail and avoid careless mistakes | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 2. | Sustain attention on tasks or play activities | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 3. | Listen when spoken to directly | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 4. | Follow through on instructions and finish school work or chores | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 5. | Organize tasks and activities | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 6. | Engage in tasks that require sustained mental effort | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 7. | Keep track of things necessary for activities | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 8. | Ignore extraneous stimuli | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 9. | Remember daily activities | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 10. | Sit still (control movement of hands or feet or control squirming) | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 11. | Stay seated (when required by class rules or social conventions) | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 12. | Modulate motor activity (inhibit inappropriate running or climbing) | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 13. | Play quietly (keep noise level reasonable) | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 14. | Settle down and rest (control constant activity) | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 15. | Modulate verbal activity (control excess talking) | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 16. | Reflect on questions (control blurting out answers) | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 17. | Await turn (stand in line and take turns) | _____ | _____ | _____ | _____ | _____ | _____ | _____ |
| 18. | Enter into conversations and games without interrupting or intruding | _____ | _____ | _____ | _____ | _____ | _____ | _____ |

## 2. Biological bases of ADHD and selection of candidate genes

In our collaborative research program, we have investigated genetic and non-genetic biological bases of ADHD. Some of our initial genetic studies were adoption studies, which set the stage for the more recent molecular genetic studies. In our first study [4], we discovered that adoption was over represented in ADHD samples. This led to an family study [17] to evaluate the similarity of ADHD children to their biological relatives and controls. Based on symptom ratings of ADHD as well signs of minor physical anomalies (e.g. wide-set eyes, low-set ears, etc.) as phenotypic marker of a 'latent trait' we proposed a genetic model in which both phenotypes derived from a common factor that was transmissible. Even though we provided evidence in favor of a single gene for ADHD, our model also predicted that less than half (43%) of the cases with ADHD would carry the gene for the latent trait. Thus, a high percentage might have an alternative (genetic or environmental) etiology that could mimic the presumed genetic cause and produce 'phenocopies' with non-genetic bases, such as fetal distress that selectively damaged striatal dopamine neurons [19]. Subsequent investigations [18] of quantitative assessment of craniofacial anomalies supported the theoretical view that both genetic and non-genetic biological factors play important roles in the etiology of ADHD. To estimate the strength of the genetic influences on ADHD, we obtained ratings on the SWAN scale in the Australian twin study [20]. The correlation of SWAN ratings was higher for monozygotic twins than for dizygotic twins, which confirmed an established finding (see e.g. Stevenson [21]) that heritability ($h^2$) of ADHD is high. In line with recent twin studies of clinical populations [22], when we used the SWAN to assess the full range of normal variation in the population, we [20] found that heritability was greater for the dimension of Attention ($h^2 = 0.9$) than for Hyperactivity-Impulsivity ($h^2 = 0.5$).

Adoption and twin study methods can be used to establish that genetic bases of a disorder exist, but they do not indicate which specific genes are involved or their location in the human genome. Methods from molecular biology are necessary to find specific genes that may play a causative role in a disorder. Two approaches are commonly used to accomplish this: a candidate gene approach [23] to consider a particular gene and a genome scan approach [24] to consider all possible genes. Since there are so many genes and the risk of false positive findings is so high, the candidate gene approach is generally not favored [23]. However, in the ADHD area this approach has been remarkably successful [25], probably because the selection of candidate genes was based on sound neuroscience theory.

In our initial molecular genetic studies of ADHD, we used two theories to select dopamine genes as candidates: (1) the dopamine deficit theory of ADHD [14], a pharmacological theory based on the efficacy of stimulant medications (dopamine agonists) to treat the disorder; and (2) the neuroanatomical network theory of ADHD [6], a neuropsy-

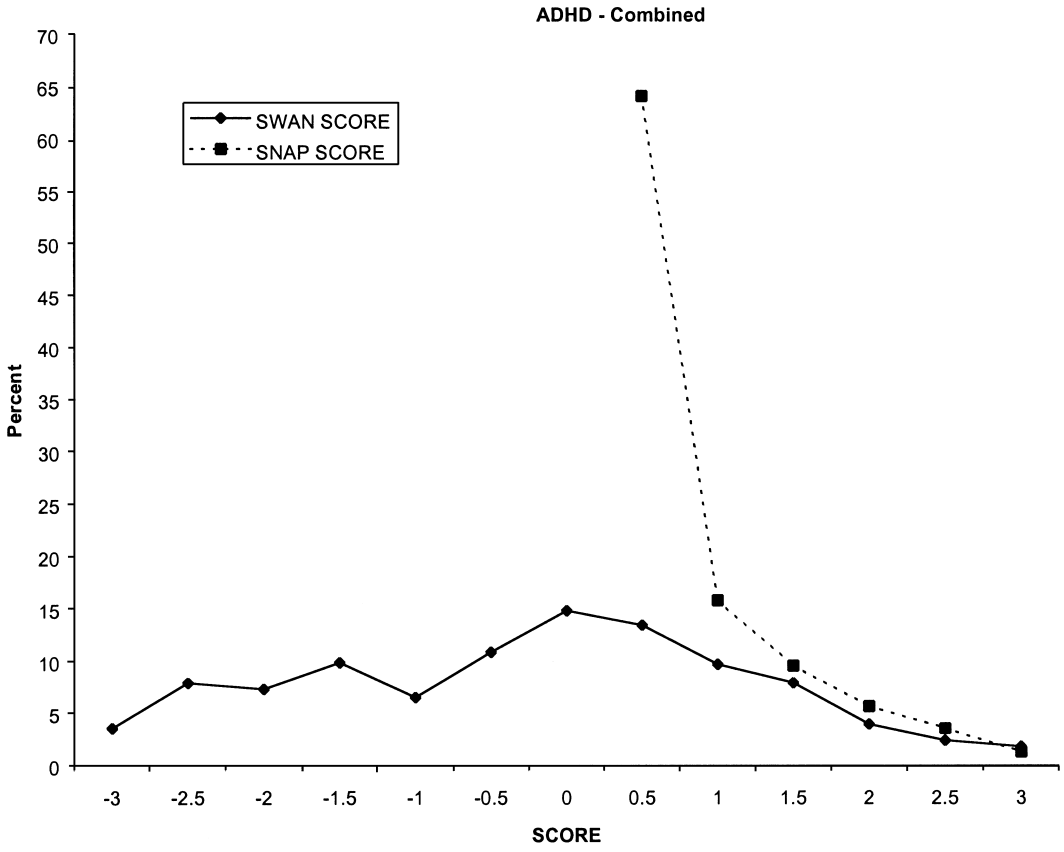


Fig. 1. School-wide ratings (population norms) for the SNAP (ADHD as Psychopathology, $n = 847$) and the SWAN (ADHD as a Dimension Encompassing Normal Behavior, $n = 506$).

chological theory based on the cognitive deficits that characterize children with this disorder. These theories of ADHD influenced the selection of two dopamine genes as candidate genes for the initial molecular genetic studies of ADHD: (a) the dopamine transporter (DAT) gene located on chromosome 5p15.3 [26] and (b) the dopamine receptor D4 (DRD4) gene on chromosome 11p15.5 [7]. The polymorphism of interest in both of these candidate genes is based on a variable number of tandem repeats (VNTR).

The DAT gene has a 40-base-pair (bp) VNTR in the 3′ untranslated region of the gene [27]. In the human population, the primary allelic variants have 9 or 10 repeats of this 40-bp sequence (denoted as DAT.9 and DAT.10). The allele frequencies vary across ethnic groups [27], but in several studies of Caucasian populations the allele frequencies were documented to be about 0.23 for the DAT.9 allele and 0.76 for the DAT.10 allele [28]. Since a primary mechanism of action of methylphenidate is the inhibition of reuptake of DA [29], the DAT gene is a logical candidate based on the site-of-action strategy. In a family-based control association study, Cook et al. [26] investigated parent-to-child transmission rates of the DAT alleles, and reported that an increased prevalence (0.85) and transmission (0.60) of the most prevalent 10-repeat allele in a sample of 119 ADHD children. Multiple replications provide strong empirical support for the DAT gene as a candidate gene for ADHD (see Ref. [28] for a review).

The DRD4 gene has a 48-bp VNTR in the coding region of the gene in the third exon (see Fig. 2, adapted from Seeman et al. [30]). The polymorphism (from 2 to 10 repeats, denoted as alleles D4.2 to D4.10) produces differences across individuals in the size of an important region of the receptor (the third intracellular loop which couples to G-proteins and mediates post-synaptic effects). In humans the

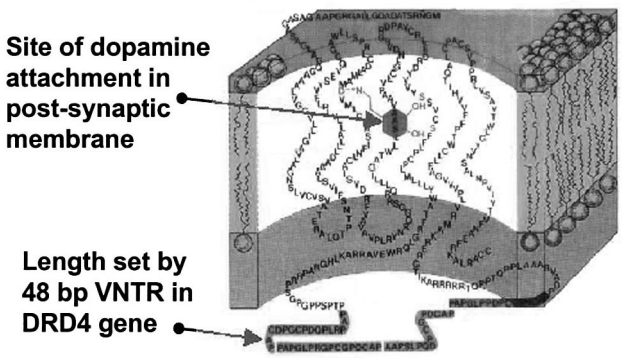


Fig. 2. The dopamine type 4 receptor showing structural variation determined by VNTR in Exon 3 of the DRD4 gene.

allele frequencies of DRD4 vary across ethnic groups [31]. In a sample of 150 unrelated Caucasians [32], the allele frequencies were 0.10 (D4.2 allele), 0.67 (D4.4 allele), 0.12 (D4.7 allele), and 0.11 (other D4 alleles).

Our collaborative research program generated the first two reports of an association of the DRD4 gene with ADHD (see Fig. 3). In the first study [7], a population-based association study of 39 children diagnosed with ADHD, we observed that the allele frequency (0.28) of the DRD4 alleles in the ADHD group was higher than the expected frequency in an ethnically matched control group (0.12). Even though this was statistically significant ($P < 0.04$), the finding was suspect since the case-control design did not protect against the feared artifact of population stratification on some unknown factor [23]. In our second study [8], we used a family-based association design to evaluate 119 ADHD probands. We obtained DNA from 136 of their parents, and in 52 families we had DNA on complete proband-parent trios. Since only 17 of these 52 parents were heterozygous for the 7-repeat allele, this sample was considered too small to apply the Transmission Disequilibrium Test (TDT), however, the Haplotype Relative Risk (HRR) test was significant at $P < 0.035$. As shown in Fig. 3, the 7-repeat allele frequency in the ADHD sample (0.27) matched the allele frequencies from the case-control study [7] and the allele frequency (0.17) for the HRR control group (defined by the non-transmitted parental alleles) was lower. Even though this study used better methods (the family-based association design) and confirmed the results of our first study, the findings were still suspect due to restricted size of the informative sample of this study.

## 3. Replication of candidate gene studies

Usually the initial positive findings that emerge from candidate gene studies are not replicated [23], but in the studies of the DRD4 gene many (but not all) of subsequent studies replicated the initial finding of an increased prevalence of the 7-repeat allele in children with ADHD. For example, Smalley et al. [34] reported the results of independent family-based association study at UCLA. In 133 families with 220 affected probands, 129 'informative' trios were collected, so the TDT statistic could be applied. In these cases, 60% of the 7-repeat alleles were transmitted, which was significantly ($P < 0.03$) greater than the expected 50% under the null hypothesis of the TDT. This provided a convincing replication of our initial findings of association of ADHD with the DRD4 gene due to an increased frequency and biased transmission of the 7-repeat allele. A recent formal meta analysis (Faraone et al. [35]) of the growing literature (seven case control studies and 14 family-based studies) concluded there was a "…statistically significant association between ADHD and the 7-repeat allele of DRD4", with a relative risk of 1.9 ($P < 0.00000008$) for seven case-control studies (four positive and three negative) and 1.4 ($P < 0.02$) for 14 family-based studies (nine positive and five negative). This literature may reflect "…a major achievement in psychiatric genetics: an association finding which has been observed in an overwhelming majority of attempts at replication" [25].

The studies that do not replicate the ADHD-DRD4 association are interesting. Two of the recent non-replications (not included in the meta-analysis by Faraone et al. [35]) are especially noteworthy, because they come from the ongoing studies at UCI [8] and UCLA [34], which now have accumulated much larger samples. Sunohara et al. [36] reported a significant TDT for 88 families in an independent sample from Toronto ($P < 0.045$), as well as for an expanded UCI sample of 111 families ($P < 0.028$). However, in the new sample of 59 families from our research program at UCI, there was no evidence of association (an equal number of the parental 7-repeat allele fell into the transmitted and non-transmitted categories). Similarly, McCracken et al. [37] reported on an expanded UCLA sample (from 220 to 371 probands). The addition of 151 trios rendered the prior significant finding [34] non-significant ($P < 0.297$). These two follow-up reports may indicate that some subtle effects related to recruitment may be operating, such that the later subset of subjects identified in a study may be less likely to show the ADHD–DRD4 association than the earlier subset entered at the beginning of the study.

Hawi et al. [38] failed to replicate the DRD4–ADHD association in family-based study with an Irish sample. They reported a higher 7-repeat allele frequency (25.6%) in an Irish control group compared to their ADHD group (24.2%). They questioned the low 7-repeat allele prevalence (about 12%) in the control groups in several studies in the United States (e.g. [5,40,41]) and suggested that the positive findings in these case-control studies might be artifacts, due "...to the low frequency of 7-repeat alleles in their control samples". Other studies support this view. For example, in the case-control study by Castellanos et al. [39], the 7-repeat allele frequency did not differ for the ADHD and control group, which had a high frequency of the 7-repeat allele (0.21). Also, two reports of samples from Israel [42,43] describe failures to replicate, but in both cases there was a high prevalence of the 7-repeat allele prevalence in an HRR control group (24.5%). However, other studies do not support this view, and may indicate that the prevalence in the Irish control group is higher than expected for a sample that is primarily Caucasian. For example, Holmes et al. [44] reported a relatively low (12.8%) prevalence of the 7-repeat allele prevalence in a large ($n = 442$) British control group, which matches the values from the United States studies criticized by Hawi et al. [38]. Studies of the DRD4 gene and novelty seeking provide some additional observations about the prevalence of the 7-repeat allele reported to be about 15% in non-clinical samples, from Israel (in 124 adults, Epstein et al. [45] reported about 14% and in a sample of 81 infants, Esptein et al. [46] reported about

17%) and from Finland (in 190 adults, Ekelund et al. [47] reported about 14%). Thus, the issue about the expected prevalence of the 7-repeat allele in the non-clinical (control) population remains unsettled.

## 4. The 7-present and 7-absent genotypes and cognition

Based on the in vitro studies by Asghari [48], one of our initial assumptions about the DRD4 gene was that the length of the 48-bp VNTR in exon III (see Fig. 2) would produce variation in the sensitivity of the D4 receptor to endogenous dopamine [6,28,33]. Even though additional in vitro data has resulted in a modification of this view [49], we speculated that the long (e.g. 7-repeat) allele might produce a subsensitive D4 receptor compared to the shorter 2-repeat and 4-repeat alleles, and that this would result in a greater dopamine deficit and a more severe form of ADHD in individuals with a 7-repeat genotype. Our initial case-control study [7] supported this hypothesis: the subgroup of ADHD cases with the 7-present genotype had a higher symptom-count than the subgroup with the 7-absent genotype.

In a recent study [50], we extended the evaluation of the DRD4 genotypes by evaluating subjects at two levels (behavioral and cognitive) of analysis based on the causal modeling approach suggested by Morton and Frith [51]. To assess ADHD symptoms at the behavioral level we used the SNAP rating scale (see Table 1), and to assess possible deficits at the cognitive level we used performance on neuropsychological tasks designed to place demands on the attentional networks proposed by Posner and Raichle [5]: the Stroop task, the Generate-Read task, and the Stop task. We evaluated 44 ADHD children and 21 control children. We obtained DNA from 32 of the ADHD subjects, and 41% had the 7-present genotype and 59% had the 7-absent genotype. As expected, both the 7-present subgroup and 7-absent subgroup differed dramatically from the control group on the behavioral SNAP ratings of ADHD severity. On measures of cognitive performance we observed the opposite of what we predicted (see Fig. 4). The 7-present subgroup did not differ from the control group in terms of speed or accuracy of performance, but the 7-absent subgroup did show a deficit (slow and inaccurate performance) on the tasks selected to impose demands on the alerting and executive control attentional networks.

Thus, our results suggest that the 7-repeat allele may be associated with a partial syndrome without a cognitive deficit instead of the full syndrome. Based on the pattern of performance (slow and variable responding) in the 7-absent subgroup, we noted similarities with the concept of minimal brain dysfunction [52], which some attribute to environmental causes such as fetal maldevelopment [19]. In the framework of evolutionary biology [53–55], the genetic form of ADHD may be due to an 'environmental mismatch' associated with the demands of modern society. The individuals with the genetic form of ADHD may posses a valuable personality trait such as novelty seeking [45] that may have been beneficial in the distant past, but in the present may produce impairment. For example, Chen et al. [56] evaluated the migration history of different ethnic groups, and showed that migration distance was highly correlated with the prevalence of the 7-repeat allele in these groups. Thus, the 7-repeat allele may have played a role in the gene flow 'out of Africa' [57] and the spread of the human population into the New World.

## 5. Allele variation of the DRD4

Most studies of the association of ADHD with the DRD4 gene have considered all 7-repeat alleles to be the same. However, several lines of research indicate this is not so. One way that 7-repeat alleles may vary is due to parental imprinting (see Weiss [58]), which occurs when the function of an allele depends on whether it was inherited from the mother or the father. In a study of the ADHD–DRD4 association in adults, Muglia et al. [59] suggested that the increased transmission of the 7-repeat allele may be largely

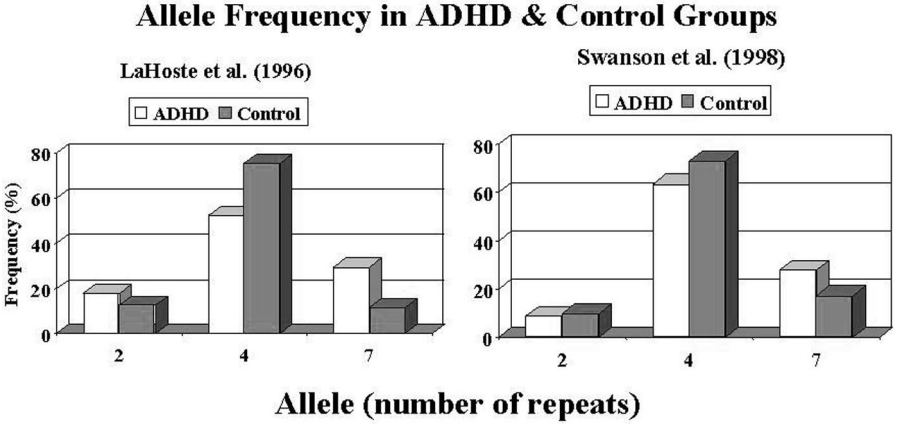


Fig. 3. DRD4 allele frequencies for ADHD and control groups.

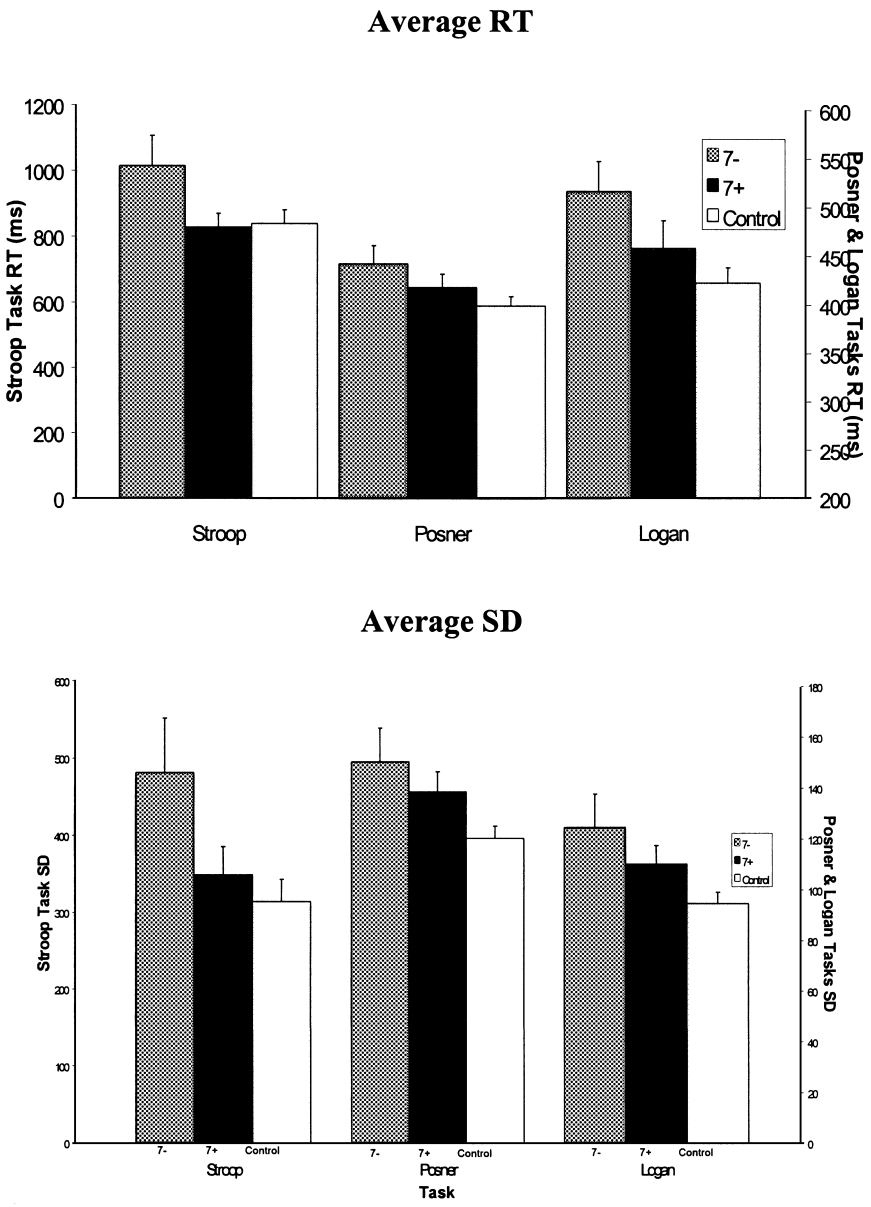


Fig. 4. RT and SD for 7-present, 7-absent, and control groups.

of maternal origin. These studies suggest that the parental origin of alleles should be evaluated to check for imprinting.

Another way that 7-repeat alleles may vary is how they occur in combination with other alleles in the same chromosomal region (i.e. as a haplotype). Barr et al. [60] investigated this in an analysis of haplotypes based on three additional polymorphic sites in the DRD4 gene. One was a 120-bp repeat 1.2 kilobases upstream to the transcription start site. The two others were single nucleotide polymorphisms (SNPs) defined by a C-to-T change at 521 bp and a C-to-G change at 616 bp before the start site. These polymorphisms are not in coding regions of the DRD4, but they may still influence the phenotype by altering the level or rate of transcription. In this study, the most common DRD4 haplotype was defined by the combination of the 7-repeat allele of the 48-bp polymorphism, the 2-repeat allele of the 120-bp polymorphism, the T allele at −521 bp, and the C allele at −616 bp (the '7-2-T-C' haplotype). Barr et al. [60] reported a biased transmission (21 transmitted vs. 10 non transmitted) for this haplotype in ADHD children. McCracken et al. [37] reported the results of a similar haplotype study of DRD4 based on the 48-bp 7-repeat and the 120-bp 2-repeat, with slightly biased transmission (66 transmitted vs. 51 non-transmitted) of the '7-2' haplotype that was not statistically significant ($P = 0.165$). These studies also suggest that the uniqueness of the 7-repeat allele may depend on the context of other polymorphisms in the DRD4 gene.

A third way that the 7-repeat alleles may differ is in terms of the actual DNA sequence of the allele. In Fig. 2 the third loop of the exon III polymorphism is depicted by amino

acids that are coded by triplicates of the bases (CCC = proline, GCG = alanine, CGC = arginine, CTC = leucine, CAG = glutamine, GAC = aspartic acid, TGC = cysteine, GGC = glycine, AAC = asparagine, etc.). For example, the 16-amino-acid sequence that specifies 48-bp sequences in the first position is Pro-Ala-Pro-Arg-Leu-Pro-Gln-Asp-Pro-Cys-Gly-Pro-Asp-Cys-Ala-Pro. Note that the 48-bp repeat is 'imperfect' [61] – not all 48-bp sequences are the same. For example, the last 48-bp sequence, which is specified by Pro-Ala-Pro-**Gyl**-Leu-Pro-**Pro**-Asp-Pro-Cys-Gly-**Ser**-**Asn**-Cys-Ala-Pro, differs from the first 48-bp sequence at four sites that change the amino acid sequence (shown in bold). Internal 48-bp repeats are more variable across individuals than the first and last 48-bp sequences of this polymorphism [61,62]. By sequencing the DRD4 gene in over 500 chromosomes, Moyzis [63] has confirmed over ten different 7-repeat alleles. If this polymorphism plays a causative role in ADHD, then it is possible (or likely) that specifying just the length polymorphism defined by the 48-bp VNTR will not be sufficient to capture important features of the DRD4 gene that may be associated with the refined ADHD/HKD phenotype. For example, the longer length may be more likely to harbor an abnormal 48-bp sequence, and it (rather than length) may have an effect on the phenotype. In our current project based on sequencing the DRD4 gene we have not yet determined the significance of the 7-repeat allelic variation, but we have confirmed that at the sequence level not all 7-repeat alleles are the same.

## 6. Other genes associated with ADHD

It is assumed that ADHD is a complex genetic disorder, with multiple genes combining to produce the phenotype. A common assumption for complex disorders is that many different combinations of alleles could produce the same phenotype. Due to a 'many to one' mapping of genotype to phenotype, it may not be possible to identify a clear genetic 'cause' as with simple Mendelian disorders [64]. One strategy is based on the assumption that the number of contributing 'risk' alleles present will increase the severity of the disorder, so comparison of the extremes of the population distribution will provide subgroups that differ in alleles of many of the genes that affect the phenotype. In our research program, we developed the SWAN rating scale (see Table 2) to allow for this type of selection. The selection from the 'ill end' of the distribution is possible based on rating scales of psychopathology (such as the SNAP), but selection from the 'well end' is not, due to the truncated assessment of normal behavior. The use of the SWAN (see Fig. 1) avoids this truncation.

The initial stages of the search for other genes associated with ADHD has generated several papers in the literature. Several investigators have extended the search to investigate other catechalominergic genes. For example, in a recent issue of *Molecular Psychiatry* [65], candidate gene studies of ADHD were reported for the DRD2, DRD3, and DRD5 receptor genes, as well as for genes related to the enzymes MAO and COMT. As recommended by Crowe [23], until multiple replications emerge for these candidate genes, a discussion of the significance of the isolated positive findings seems premature.

Studies of non-catecholaminergic genes have also been conducted, based on animal models of ADHD. One interesting line of research is based on the Coloboma mouse model of ADHD [66], which is related to a mutation in the SNAP-25 gene that produced a mouse with hyperactivity that is responsive to amphetamine. In the Toronto sample, Barr et al. [67] found an association between ADHD and the SNAP-25 gene. Another line of research is based on the serotonin hypothesis of ADHD [68], which led Quist et al. [69] to investigate and report an association of the HTR2A receptor gene and ADHD.

Not all candidate genes are expected to generate findings that will replicate. For example, the plausible theory that genetic variation in COMT (an enzyme important in the metabolism of dopamine) may play a role in the manifestation of ADHD was supported in an initial candidate gene study [70], but this finding was not replicated by the same group in an independent sample [71] or by another group who attempted to replicate [72]. This reinforces the advice of Crowe [23] to require multiple replications before accepting the findings of candidate gene studies.

## 7. Conclusions

The initial molecular genetic studies of ADHD used the candidate gene approach, and the application of the pharmacological 'site of action' theory related to treatment with stimulant drugs and the neuroanatomical network theory related to attentional deficits resulted in a focus on dopamine genes. This strategy has been successful, and two confirmed associations (with the DAT and DRD4 genes) have been documented.

The next stage of providing a fine-grained analysis of these associations is underway. The complexities of haplotype of a given gene (e.g. the DRD4) or combinations of multiple genes are emerging in the literature. These pioneering studies point the way to the next stages of research to pin down causative factors that are expected to exist.

Even though the candidate gene approach has been very successful in the ADHD area [25], many of the initial findings are expected to be false positive associations [23]. The alternative approach, a genome scan to identify chromosome regions involved in ASHD, followed by intensive search in those regions where the locus of an associated gene has a high prior probability, is being pursued by multiple research groups. No report of a genome scan has yet been published, but in the near future the reports of findings from multiple genome scans are expected. The use of pooled

samples may be essential to obtain a sample size large enough for this task. An ADHD Molecular Genetics Network [73] has been formed for this purpose, which has spurred the development of operational definitions for a common assessment of cases across multiple sites and methods [74]. These represent concrete steps in the direction of large-scale collaborations to elucidate the complex molecular genetic bases of ADHD.

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