Genetics of Childhood Disorders: XXII. ADHD, Part 6: The Dopamine D4 Receptor Gene

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The neurotransmitter dopamine is released at the presynaptic terminal, diffuses across the synaptic cleft, and binds to specific dopamine receptors on the plasma membrane of the postsynaptic terminal. Five distinct dopamine receptors have now been cloned, each produced by a different gene (D1–D5 receptors). These different genes presumably arose by gene duplication millions of years ago, and significant differences in their signaling properties have evolved over time. Two general classes have emerged based on pharmacological studies: the D1 and D5 receptors generally transmit stimulatory signals, whereas the D2–D4 receptors are inhibitory.

In addition to these five separate dopamine genes, individual receptors may vary to some extent between different populations and individuals. These differences ultimately are based on differences in the nucleotide sequences that also emerged over millions of years of evolution. The differences in nucleotide sequences can now be directly determined in the laboratory by sequencing of the gene in question. Obviously, when the sequences in a gene from two individuals are compared, differences in the sequences will emerge. These changes in sequences are termed *polymorphisms*. Polymorphisms may occur as single base substitutions, as deletions or insertions, or as multiple repetitions of a particular sequence of base pairs (bp). This last type of polymorphism was the first polymorphism discovered within the *DRD4* gene, and it turned out to be a 48-bp repeated sequence.

The interest in polymorphisms arose when it was realized that they could be used as markers to help localize genes that cause or contribute to the expression of a particular illness. For example, if a particular polymorphism is found to be passed on to the next generation and all individuals who received that polymorphism develop a specific disease, then the investigators would have considerable evidence that either that particular gene or one that lies very close to it is responsible for the symptoms. This is in fact the basis for genetic linkage studies (Pauls, 1999).

Polymorphisms may, or may not, change the functional properties of the protein in which they occur. Whether the enzymatic properties of a protein are affected depends on where the nucleotide changes occur within the gene. Changes to the coding region of a gene are more likely to have functional consequences, as many of these nucleotide changes will alter the amino acid sequence of the protein.

Changes outside of the coding region of a gene are less likely to have an effect on the enzymatic activity as they are less likely to change its amino acid structure. There are times, however, when even a polymorphism outside of the coding region has a dramatic effect on the protein's functioning. This occurs, for example, when the polymorphism arises within the promoter region and affects the amount of transcription, either increasing or decreasing the amount of message produced. Another example is when the polymorphism falls within an intron and alters the nucleotide sequence that regulates the correct splicing of the RNA message.

Since the cloning of the gene for the dopamine D4 receptor (*DRD4*) in 1991 (Paterson et al., 1999), it has received considerable attention in genetic studies. Much of the original research focused on the possible role of the D4 receptor gene in schizo-

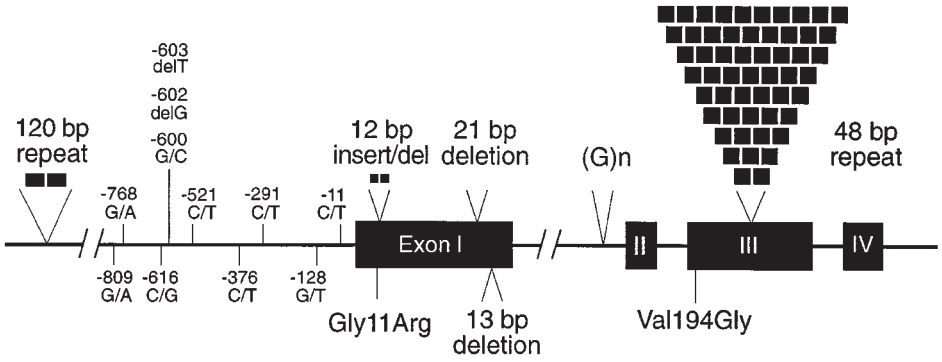

Fig. 1 Schematic showing the location of polymorphisms and mutations in *DRD4*.

phrenia because of the high affinity of the D4 receptor for the atypical antipsychotic clozapine (Paterson et al., 1999). Since then, *DRD4* has been investigated in a number of other behavioral phenotypes (see for review, see Van Tol et al., 1992) including personality traits, bipolar affective disorder, alcohol and drug abuse, and most recently attention-deficit/hyperactivity disorder (ADHD), the focus of this review.

A number of polymorphisms have been identified in the exons and introns of *DRD4,* including a 12-bp repeat in the first exon and a 48-bp repeat in exon III (Fig. 1). Less common DNA variants have also been identified in the coding region of the gene including a 21-bp deletion and a 13-bp deletion both located in exon 1, a substitution of arginine for glycine at position 11, and a substitution of glycine for valine at position 194 (for summary, see Van Tol et al., 1992). Additional polymorphisms have been identified in the region upstream of the coding sequence including a 120-bp repeat located approximately 1.2 kb upstream of the first codon, as well as several single nucleotide polymorphisms (LaHoste et al., 1996; Lichter et al., 1993). The majority of genetic studies have used the 48-bp repeat polymorphism located in the third exon mainly because it was the first polymorphism identified and encodes for relatively large changes in the protein sequence (Swanson et al., 1998). The number of repeats varies from 2 to 10 in the human population, and there is sequence and amino acid variation within the repeats as well (Rowe et al., 1998). Substantial genetic diversity of the repeats across different ethnic groups has been documented (Van Tol et al., 1992).

In 1996, two studies reported an association between the personality trait of novelty-seeking and the allele of the *DRD4* with 7 copies of the 48-bp repeat. This finding has not been replicated by other groups and is still considered controversial. The issues concerning these reports have been previously covered in detail (Van Tol et al., 1992) and will therefore not be reviewed here.

Regardless of whether or not a genetic relationship between *DRD4* and novelty-seeking exists, these initial reports led to further investigations of *DRD4* as a susceptibility factor for ADHD. The first study used a case-control design and found an increased frequency of the 7-repeat allele in the ADHD sample compared with an ethnically matched control group (Smalley et al., 1998). Those authors subsequently collected proband/parent trios and the same allele was found to occur at a higher frequency in probands than in the nontransmitted alleles of the parents (Faraone et al., 1999). This type of family-based association study markedly reduces the risk for false-positive associations as the control group are the parents of the probands and not another group of potentially ethnically diverse individuals in which normal polymorphisms are routinely found.

Further support for *DRD4* in ADHD has come from a number of additional published studies using different sample ascertainment strategies and statistics (Castellanos et al., 1998; Jovanovic et al., 1999). Several different statistical approaches were used by Rowe and colleagues (1998) to investigate the genetic relationship between *DRD4* and ADHD. Using a case-control approach, they found that the 7-repeat allele occurred more frequently in children with the ADHD-inattentive subtype than in controls. Using family data, the authors also examined the transmission of alleles with both a categorical and a quantitative transmission disequilibrium test (TDT). No significant evidence was found for biased transmission of the 7-repeat allele when a diagnosis of ADHD was made by either a categorical or a quantitative approach. However, a positive correlation was reported for inattentive symptoms and the 7-repeat allele when a quantitative TDT was used. The authors also investigated genetically discordant sibling pairs and observed that the sibling with a greater number of 7-repeat alleles displayed more inattentive symptoms than the respective cosibling with fewer 7-repeat alleles. A larger sample size would help to further clarify the relationship of *DRD4* to the inattentive versus the hyperactive subtype.

Using a sample of 27 ADHD children and their parents that was collected through parents referred with adult ADHD, Faraone et al. (1999) reported the biased transmission of the 7-repeat allele compared with the 4-repeat allele ($\chi^2 = 7.4$, $df = 1$, $p = .007$). In contrast, Smalley et al. (1998), using genotypes from 133 families consisting of 49 families with a single ADHD proband and 84 with at least one additional affected sibling, found no significant evidence for identity-by-descent sharing of *DRD4* 48-bp repeat alleles. The investigators then stratified the informative meioses into those with and without the 7-repeat allele. When the data were analyzed in this way, a trend emerged ($p = .07$) with increased allele-sharing among the sibling pairs from parental meioses in which the 7-repeat was present. An additional study also reported little evidence for an association. This study used a case-control design with 41 children with ADHD and 56 controls group-matched for ethnicity and sex.

In addition to the published studies, two unpublished studies out of Toronto support the association. The first study used two samples ascertained through probands with adult ADHD. Results were significant for a case-control sample of 66 cases with ethnically matched controls ($\chi^2 = 5.65$, $p = .01$). There was a trend for biased transmission of the 7-repeat allele in a sample of 44 nuclear families with an ADHD adult proband, but this was not significant ($\chi^2 = 2.00$, $p = .15$). The results were significant after the samples were combined ($N = 110$, $z = 2.68$, $p = .003$).

Our group in Toronto has accumulated further support for biased transmission of the 7-repeat allele by using the TDT test in an independent sample of children. This sample of 107 families was ascertained through child ADHD probands collected at the Hospital for Sick Children. Using a one-sided test we

observed biased transmission of the 7-repeat allele ($\chi^2 = 2.882$, $p = .045$).

While a number of studies thus far support *DRD4* as a genetic susceptibility factor in ADHD, the findings are not robust. It is clear that the 7-repeat allele is neither necessary nor sufficient to cause ADHD and that this is not a straightforward relationship. Therefore, it is still imperative to consider that ADHD is a complex trait and much more evidence needs to be accumulated before we can be conclusive about *DRD4* or any gene in this disorder.

Working under the assumption that there is some type of relationship between the *DRD4* and ADHD, we are left with trying to find a biological explanation for these observations. Functional differences in the intracellular signaling system have been reported for the 48-bp repeat alleles, and the 7-repeat allele may be less sensitive to endogenous dopamine. One could speculate that a blunted response of the 7-repeat allele to dopamine is consistent with the ameliorative effects of methylphenidate treatment. In other words, methylphenidate raises synaptic dopamine levels, thus compensating for the blunted response of the receptor. However, the functional differences between the repeats are small and it is not yet clear how these subtle effects could be related to the phenotype of ADHD.

A second possible explanation is that an additional as yet unidentified DNA variant in linkage disequilibrium with the 7-repeat allele is contributing to the phenotype. My laboratory has been investigating the second possibility, and we have some interesting preliminary findings that suggest that the susceptibility may be more complicated than the simple inheritance or not of the 7-repeat allele as a risk factor.

To understand more about the molecular basis of the finding, we examined the inheritance of other polymorphisms in the gene and also the inheritance of the groupings of alleles on the chromosome (the haplotypes). We assembled the haplotypes of individuals with the 48-bp repeat polymorphism, the mononucleotide repeat in the first intron, and the 12-bp repeat in the first exon. We observed biased transmission of two haplotypes formed from these three polymorphisms. One of these was the only common haplotype containing the 7-repeat allele ($\chi^2 = 4.900$, $df = 1$, $p = .027$).

The most surprising finding from the study of the haplotypes was the observation that one haplotype containing a 4-repeat allele was *not* transmitted to the affected probands more often than expected by chance. If the risk conferred by *DRD4* is simply related to the inheritance of the 7-repeat allele, then there should be no bias as to what allele is not transmitted. That is, for a two-allele marker, if one allele is preferentially transmitted, then the other allele will not be transmitted. For a system with more than one allele (in this case, more than one haplotype), then the nontransmitted allele should be distributed across all of the other alleles. The finding that one haplotype is preferentially not transmitted suggests that this haplotype may provide some type of protective role. While this is an interesting finding, the observation must be extensively replicated before any conclusion can be made.

We also searched for other known DNA variants that result in changes in the protein sequence including two previously reported deletions, a 13-bp and a 21-bp deletion in the first exon (see Fig. 1). We did not observe either of these two deletions in our sample, indicating that these deletions are not responsible for the reported association. We also tested for linkage to the 12-bp insertion/deletion polymorphism in the first exon. We did not observe biased transmission of the alleles of this polymorphism.

Where do we take this research from here? A number of investigators are concentrating on dissecting the phenotype in relation to this gene, in particular the components of the phenotype with respect to the inheritance of the 7-repeat allele. If the number of 48-bp repeats is not responsible for the phenotype, then identification of the DNA variant responsible for this finding is necessary and the analysis of the components of the phenotype in relationship to this allele may be premature. One line of inquiry that has not thus far been pursued is the sequence variation within the repeats. It is possible that different sequence variations or arrangement of variants within the repeats may be more closely associated with the phenotype. The identification of the responsible variant may be difficult, however, as it is likely that there are a number of common variants found in individuals with and without ADHD and proving causality is difficult with a common complex phenotype.

Much more work is needed to understand the molecular underpinnings of the *DRD4* and ADHD findings, including the relationship of the genotype to the phenotype. Investigating the interaction with other genetic and environmental susceptibility factors and the relationship to comorbid disorders should also be the goal of future research.

WEB SITES OF INTEREST

http://www.utexas.edu/research/asrec/dopamine.html
http://www.tocris.com/dopamine.htm
http://www.add.org/
http://medstat.med.utah.edu/calendar/block4.html/ppt_parkinson_162/

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Accepted July 18, 2000.

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