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BRIEF REPORT

Association of DRD4 with attention problems in normal childhood development

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Several previous studies found an association of clinically diagnosed attention deficit hyperactivity disorder with long alleles of a variation in the DRD4 dopamine receptor gene exon III coding sequence. We evaluated the DRD4 polymorphism in a non-clinically selected sample of children for whom maternal reports of attention problems were available at 4 and 7 years of age. There was a significant elevation in attention problem scores in children carrying DRD4 long alleles that accounted for 3–4% of total variation at each age and for 5–7% of the temporally stable component of the phenotype. Our results show that the DRD4 gene influences normal as well as pathological attention processes, and the results highlight the utility of longitudinal measurements in psychiatric genetics. Psychiatr Genet 11:25–29 © 2001 Lippincott Williams & Wilkins.

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INTRODUCTION

The D4 dopamine receptors (DRD4s) belong to the D2-group of dopamine receptors and are predominantly expressed in a restricted set of dopamine-rich limbic areas that are involved in cognition and emotion. Both pharmacological and genetic experiments suggest that D4DRs play important roles in attentional, motivational, and exploratory neurobehavioral processes. The human DRD4 gene, which is located on chromosome 11p15.5, contains a highly polymorphic 48 base pair variable number of tandem repeats sequence in exon III. This polymorphism, for which the four- and seven-repeat alleles are most common, lies in the third cytoplasmic loop of the receptor and has been reported to have modest effects on both ligand binding and receptor-mediated modulation of intracellular cyclic AMP levels (Vantol *et al*., 1992; Asghari *et al*., 1994, 1995).

There have been numerous studies on the possible role of the DRD4 exon III polymorphism in human behavior, personality, and psychiatric disorders. The most consistent results involve attention deficit hyperactivity disorder (ADHD), a common neuropsychiatric disorder with childhood onset that is characterized by problems with attention, information processing, overactivity and impulsivity. ADHD is known to have a substantial genetic component from family, twin and adoption studies, and dopaminergic genes are reasonable candidates for this effect in view of the fact that the most common pharmacological treatment of the disorder is with drugs such as methylphenidate that alter dopamine transmission (Faraone and Biederman, 1998; Thapar *et al*., 1999). There have now been eight reports of an association between ADHD and DRD4 allele 7, which is the most common long variant of the exon III polymorphism (LaHoste *et al*., 1996; Rowe *et al*., 1998; Smalley *et al*., 1998; Swanson *et al*., 1998; Comings *et al*., 1999; Faraone *et al*., 1999; Muglia *et al*., 2000; Tahir *et al*., 2000). These positive findings have included both population-based case–control studies and within-family designs, and they have employed several different types of diagnostic criteria. One study found that the association was strongest for inattentive-type ADHD (Rowe *et al*., 1998). However, there have also been four reported failures to find association between DRD4 and ADHD (Castellanos *et al*., 1998; Eisenberg *et al*., 2000; Hawi *et al*., 2000; Kotler *et al*., 2000).

The DRD4 exon III polymorphism has also been associated with the normal personality trait of novelty seeking, with long alleles leading to higher

scores (Benjamin *et al*., 1996; Ebstein *et al*., 1996, 1997; Ono *et al*., 1997; Noble *et al*., 1998; Strobel *et al*., 1999; Tomitaka *et al*., 1999). These positive studies have used both population-based and within-family designs, various diagnostic instruments, and several different ethnic populations. Furthermore, DRD4 knockout mice exhibit a specific deficit in exploration of novel stimuli (Dulawa *et al*., 1999), and novelty seeking is analogous to ADHD in that both involve a preference for new stimuli, high activity level, and impulsiveness. However, numerous other studies have failed to find an association between DRD4 and novelty seeking or other dimensions of normal personality (reviewed in Paterson *et al*., 1999). Other studies have examined the relationship of the DRD4 gene to opioid abuse, other forms of substance abuse, and pathological gambling in adults (Kotler *et al*., 1997; Li *et al*., 1997; Mel *et al*., 1998; Comings *et al*., 1999; Franke *et al*., 2000), and to various aspects of temperament and behavior assayed by the Brazelton and neonatal behavioral assessment scales in 2-week-old and 2-month-old infants (Ebstein *et al*., 1998; Auerbach *et al*., 1999).

The present work examines the DRD4 exon III polymorphism in a group of children that are being followed as part of a longitudinal study of normal childhood development with an emphasis on temperament and social behavior (Fox *et al*., 1995, 2001; Calkins *et al*., 1996). Based on the studies already described, we predicted that children with long alleles of the DRD4 gene would have more attention problems than children with short alleles. To test this hypothesis, maternal reports of attention problems were obtained when the children were 4 and 7 years old, and buccal cells were collected for DNA preparation. The availability of phenotype data at two different ages allowed us to examine the contribution of the DRD4 gene to the longitudinal continuity of attention problems as well as at two specific points in development.

SUBJECTS AND METHODS

Subjects

The subjects of this study were 174 children (81 males, 93 females) from four cohorts of children selected during infancy and early childhood, and followed at 9, 14, 24, 48, and 84 months (Fox *et al*., 1995, 2001; Calkins *et al*., 1996). The children were primarily Caucasian and of middle-class background. All of the parents had completed high school, and a majority of the mothers and fathers were college graduates. The children were, for the most part, living with their families in or near College Park, MD, USA. Original exclusion criteria were that the child had no peri- or post-natal complications, and the child had no known neurological problems. Eleven of the children developed attention deficit disorder or ADHD between the initial ascertainment and the final examination at 7 years. Parents were briefed about the procedures and written consent was obtained before entry into the study.

Maternal report of childhood attention problems

Maternal perceptions of childhood attention problems were assessed at ages 4 and 7 with the Childhood Behavior Checklist (CBCL) (Achenbach and Edelbrock, 1981). The CBCL is a widely used 113-item checklist in which parents use a three-point scale to rate how descriptive a series of behavior problems are of their child. The CBCL yields eight narrow-band factors that index a variety of problems. The present study focused on the CBCL subscale related to attention problems, which comprises 11 items. Data were available for 161 children at age 4 and for 107 children at age 7; of these, 94 children had data available for both ages. The mean ($\pm$ sem) raw scale scores were 2.36 ± 0.16 at age 4 and 2.70 ± 0.25 at age 7. The scores were converted to Z values (normalized to have a mean of 0 and a standard deviation of 1) prior to averaging and statistical analysis.

DNA preparation and genotyping

Genomic DNA was prepared from buccal swabs (Epicentre Technologies, Madison, WI, USA). Children were instructed by an investigator or parent to collect cheek cells by rolling a buccal brush firmly on the inside of the cheek, approximately 20 times on each side. The brushes were air-dried for 10–20 min and then extracted within 3 days. DNA was prepared by absorption to a bead matrix and heat elution according to the manufacturer's instructions.

The DRD4 gene exon III polymorphism was amplified by the polymerase chain reaction, and the number of repeats was determined by electrophoresis through a 3.5% agarose gel and ethidium bromide staining (Lichter *et al*., 1993; Benjamin *et al*., 1996). Allele frequencies were 6.0% allele 2, 1.7% allele 3, 69.0% allele 4, 1.4% allele 5, 0.3% allele 6, 21.3% allele 7, and 0.3% allele 8.

Statistical analysis

Based on previous results (Benjamin *et al*., 1996), genotypes were classified into two groups: short (S) for s/s and long (L) for s/l and l/l, where s

indicates alleles with two to five repeats and l indicates alleles with six to eight repeats. Similar results were obtained when genotypes were coded according to the presence or absence of the seven-repeat allele (Ebstein *et al*., 1996) or as the sum of allele lengths. Association of dichotomized DRD4 genotype with attention problem scores was determined by one-way analysis of variance using all data available at each age (Table 1).

Pearson correlations were calculated by coding the DRD4-S genotype as 1 and the DRD4-L genotype as 2 using the 94 subjects for whom data were available at both ages (Figure 1). DRD4 effect sizes were calculated as

$$\text{DRD4a} = (\text{score in DRD4-S genotype group} - \text{score in DRD4-L genotype group})/\text{sd}_{\text{total}},$$

where sd_{total} is the population standard deviation. DRD4 exon III polymorphism-specific heritabilities were calculated as $h^2_{\text{DRD4}} = (\text{var}_{\text{DRD4}} \times E^2)/\text{var}_{\text{total}}$, where var_{DRD4} is the variance in dichotomized DRD4 genotype, E is the effect size (score in DRD4-S genotype group − score in DRD4-L genotype group), and $\text{var}_{\text{total}}$ is total phenotypic variance.

Results are reported for the entire sample, without regard to ethnic group, because there was no significant evidence for population stratification artifacts within this sample. The DRD4 effect sizes in Caucasians, the largest ethnic group ($n = 157$; 90.2%) were DRD4 = 0.41 at 4 years, 0.46 at 7 years, and 0.52 for the average as compared with 0.35, 0.40, and 0.47 for the entire sample. The corresponding Pearson correlations between DRD4 genotype and attention problems were 0.259 at 4 years and 0.183 at 7 years in Caucasians as compared with 0.233 and 0.169 for the entire sample. The average attention problem Z scores (mean ± sem) and DRD4 exon III polymorphism genotype distributions (percentage of L genotypes) after stratification by ethnic group were as follows: for Caucasians ($n = 157$), $Z = -0.03 \pm 0.10$,%L = 39; for African−Americans ($n = 6$), $Z = 0.20 \pm 0.64$,%L = 50; for Hispanics ($n = 1$), $Z = 0.30$,%L = 100; for unknown ethnic group ($n = 10$), $Z = -0.14 \pm 0.17$,%L = 40.

RESULTS AND DISCUSSION

Maternal reports of attention problems were obtained when the children were 4 and 7 years of age and analyzed for association with the DRD4 gene (Table 1). At age 4, children with one or two long alleles of the exon III polymorphism scored, on average, 0.35 standard deviations higher for CBCL attention problems than did children homozygous for short alleles of the polymorphism, a significant difference ($P = 0.03$). At age 7, there was a difference in the same direction of 0.40 standard deviations ($P = 0.04$). When the standardized scores were averaged, the difference between long and short genotypes was 0.47 standard deviations ($P = 0.02$). The corresponding DRD4 exon III polymorphism-specific heritabilities were 0.029 at 4 years, 0.038 at 7 years, and 0.053 for the average.

The availability of data at two different ages allowed us to calculate the contribution of the DRD4 polymorphism to the longitudinal continuity of attention problems from the correlations shown in Fig. 1 according to the formula:

$$h^2_{\text{DRD4:ATT4:ATT7}} = r_{\text{DRD4:ATT4}} \times r_{\text{DRD4:ATT7}}/r_{\text{ATT4:ATT7}} = 0.075$$

where $h^2_{\text{DRD4:ATT4:ATT7}}$ measures the degree to which the longitudinal continuity of attention problems between ages 4 and 7 is due to the DRD4 exon III polymorphism; in other words, $h^2_{\text{ATT4:ATT7}}$ is the DRD4 exon III polymorphism-specific heritability of the temporally stable component of attention problems. This computation was checked by a partial correlation analysis, which showed that the correlation between attention problems at ages 4 and 7 after correcting for the DRD4 gene was $r'_{\text{ATT4:ATT7}} = 0.049$, giving $h^2_{\text{DRD4:ATT4:ATT7}} = [(r_{\text{ATT4:ATT7}})^2 -$

TABLE 1. Association between DRD4 genotype and CBCL attention problems

CBCL Attention problem Z score (mean ± se)					
DRD4-S group	DRD4-L group	F	DRD4	h^2_{DRD4}	P
Age 4					
−0.139± 0.093 (n=97)	0.210 ± 0.136 (n=64)	4.79	0.35	0.029	0.030
Age 7					
−0.150± 0.111 (n=67)	0.251 ± 0.174 (n=40)	4.16	0.40	0.038	0.044
Average					
−0.173± 0.097 (n=59)	0.226 ± 0.159 (n=35)	5.16	0.47	0.053	0.025

F, One-way analysis of variance statistic; DRD4, effect size in standard deviation units; h^2_{DRD4}, DRD4 exon III polymorphism-specific heritability; P, two-sided significance level.

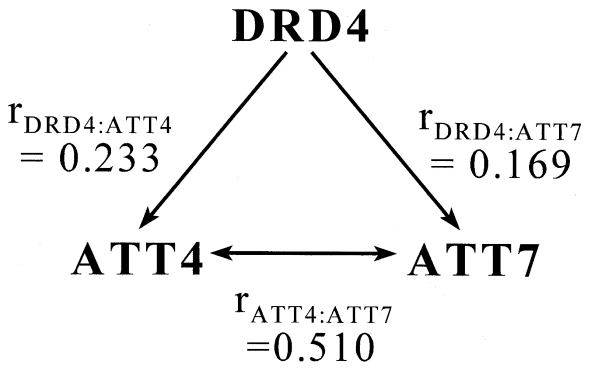

FIGURE 1. Genotype–phenotype correlations at two ages. Pearson correlations (r) were calculated for the 94 children for whom data were available at both 4 and 7 years. DRD4, Exon III polymorphism of the DRD4 gene, dichotomized as 1 for the S genotype and 2 for the L genotype; ATT4, CBCL attention problems at age 4; ATT7, CBCL attention problems at age 7.

$(r'_{ATT4:ATT7})^2]/(r_{ATT4:ATT7})^2 = 0.073$, in good agreement with the value of 0.075 calculated earlier.

Our results provide independent support for the association of attention problems with long alleles of the exon III coding polymorphism of the DRD4 gene previously reported by several groups (LaHoste *et al*., 1996; Rowe *et al*., 1998; Smalley *et al*., 1998; Swanson *et al*., 1998; Comings *et al*., 1999; Faraone *et al*., 1999; Muglia *et al*., 2000; Tahir *et al*., 2000). An important feature of the current work is that it examined an unselected group of children rather than a sample selected on the basis of having an ADHD diagnosis. In an accompanying paper, Auerbach *et al*. (2001) provide evidence for the association of DRD4 long alleles with less sustained attention in a non-clinical group of 1-year-old infants. The implication of these findings is that the DRD4 gene contributes to the full spectrum of attentional abilities rather than solely to extreme problems. It may turn out that the clinical diagnosis of ADHD represents an arbitrarily defined tail of a continuous distribution of attentional abilities rather than a discrete disease entity that is genetically 'carved at the bone'. This may help to explain why diagnosis rates for ADHD have changed so much in recent years.

A second feature of our work is that attentional problems were assessed at two different ages. As hoped, this longitudinal design was more powerful than a single measurement for demonstrating specific genetic effects on attention problems. Specifically, we found that DRD4 exon III polymorphism-specific heritability was greater for the average of the two time points (0.053) than for either time point alone (0.029 and 0.038). A path calculation indicated that this is because the DRD4 gene contributes to 7.5% of the stable component of attention problems between 4 and 7 years of age as compared with less than 4% of the variance at either age by itself. Part of the increased heritability may be because repeated measures reduce measurement error. More importantly, increased heritability is expected because genes stay the same while the environment changes. Hence, this type of longitudinal approach may be useful to identify and analyze other genes involved in psychiatric diseases.

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