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Parent-Child *DRD4* Genotype as a Potential Biomarker for Oppositional, Anxiety, and Repetitive Behaviors in Children with Autism Spectrum Disorder

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Abstract

The primary objective of the present study was to examine whether a combination of parent-child *DRD4* genotypes results in more informative prognostic biomarkers of oppositional, separation anxiety, and repetitive behaviors in children with autism spectrum disorder (ASD). Based on prior research indicating the 7-repeat allele as a potential risk variant, participants were sorted into one of four combinations of parent-child genotypes. Owing to the possibility of parent-of-origin effects, analyses were conducted separately for mother-child (MC) and father-child (FC) dyads. Mothers completed a validated DSM-IV-referenced rating scale. Partial eta-squared (ηp^2) was used to determine the magnitude of group differences: 0.01–0.06=small, 0.06–0.14=moderate, and >0.14=large. Analyses indicated that children in MC dyads with matched genotypes had the least (7−/7−) and most (7+/7+) severe mother-rated oppositional-defiant (ηp^2=0.11) and separation anxiety (ηp^2=0.19) symptoms. Conversely, youths in FC dyads with matched genotypes had the least (7−/7−) and most (7+/7+) severe obsessive-compulsive behaviors (ηp^2=0.19) and tics (ηp^2=0.18). Youths whose parents were both noncarriers had less severe tics than peers with at least one parental carrier, and the effect size was large (ηp^2=0.16). There was little evidence that noncarrier children were rated more severely by mothers who were carriers versus noncarriers. Transmission Disequilibrium Test analyses provided preliminary evidence for undertransmission of the 2-repeat allele in youths with more severe tics (p=0.02). Parent genotype may be helpful in constructing prognostic biomarkers for behavioral disturbances in ASD; however, findings are tentative pending replication with larger, independent samples.

Keywords

autism spectrum disorder; *DRD4*; oppositional defiant disorder; separation anxiety disorder; obsessions-compulsions; tic disorder

1. Introduction

Decades of research have clearly shown that raising a child with autism spectrum disorder (ASD) can be a profoundly stressful experience for parents, a task that is seriously exacerbated by co-occurring irritability, noncompliance, temper tantrums and repetitive behaviors (e.g., Benson, 2006; Hastings et al., 2005; Herring et al., 2006; Lecavalier et al., 2006; Pottie et al., 2009), and may even impact maternal cortisol levels (Seltzer et al., 2009). Although the clinical implications of dysfunctional interpersonal interactions are self-evident to experienced clinicians (e.g., Rao & Beidel, 2009), less well appreciated are their relevance for molecular biology as maternal report is often the primary or sole basis for diagnosing or characterizing neurobehavioral (endo-) phenotypes and co-occurring symptomatology. In other words, maternal genotype influences mother-child interactions, child disruptive behavior, as well as perceptions and therefore reports of behavior problems.

For a variety of reasons, few investigators have explored candidate genes for behavioral disturbances in children with ASD, but preliminary findings suggest that some common susceptibility alleles may be potential biomarkers for severity of co-occurring symptomatology in this clinical population (e.g., Brune et al., 2006; Cohen et al., 2003; Gadow et al., 2008b, 2009; Roohi et al., 2009). One example is a common 48bp variable number tandem repeat (VNTR) polymorphism within exon 3 of the D4 receptor gene (*DRD4*) located on chromosome 11. The actual number of repeats varies from 2 to 11, with the 2-, 4-, and 7-repeats being the most common. The 7-repeat allele purportedly results in less D4 receptor responsiveness (reduced dopamine binding efficiency) (Asghari et al., 1995; Cravchik et al., 2000; van Tol et al., 1992) and shows an association with response to pharmacotherapy (McGough 2005) and behavioral intervention (Bakermans-Kranenburg et al., 2008) for disruptive behaviors.

Previously, we found that youths with ASD and who had at least one copy of the 7-repeat allele obtained significantly more severe maternal ratings of oppositional defiant disorder (ODD) (ηp^2=0.10), obsessive-compulsive behavior (OCB) (ηp^2=0.08), and tics (ηp^2=0.07) than youths with two shorter alleles (Gadow and co-workers, unpublished results). In addition, there was tentative evidence (p=0.08) that 7-repeat allele carriers had more severe symptoms of separation anxiety disorder (SAD) (ηp^2=0.05), a syndrome that appears to have considerable overlap with ODD (Foley et al., 2004; Gadow et al., 2008a).

These results are particularly interesting in light of recent research with typically developing, non-ASD samples linking maternal stress, parenting, and child behavior problems. For example, there is evidence that mothers who are *DRD4* 7-repeat allele carriers (7+) are more reactive to stress and engage in less sensitive parenting than noncarriers (7−) (van Ijzendoorn et al., 2008). Moreover, it has been reported that child *DRD4* 7-repeat allele carriers are differentially more responsive to certain types of parenting behavior (e.g., Bakermans-Kranenburg & van Ijzendoorn, 2006, 2007; Bakermans-Kranenburg et al., 2008; Gervai et al., 2007; Sheese et al., 2007), have more problems with peer aggression (DiLalla et al., 2009), and are more reactive to negativistic parenting behavior, which likely contributes to the ontogeny of child aggression (Bakermans-Kranenburg & van Ijzendoorn, 2006; DiLalla et al., 2009). To date, however, no studies have examined whether parental *DRD4* genotype is associated with challenging child behaviors or if knowledge of parent genotype can be used to enhance the clinical utility of gene-behavior biomarkers in children with ASD.

Owing to the complexities of both gene-behavior associations and the biologic mechanisms that underlie behavioral variation, we examined mother-child (MC) and father-child (FC) dyads separately. Based on the aforementioned research, we predicted *á priori* that children in MC7− dyads would evidence significantly less severe symptoms than dyads with two 7-repeat allele carriers (MC7+). Planned comparisons were also conducted to determine if children who were noncarriers were rated differently by mothers who were carriers versus noncarriers (e.g., maternal genotype might directly influence or alter perceptions of mother-child interactions). To explore whether *DRD4* alleles might be involved in symptom modulation, family-based allelic transmission analyses were also conducted.

2. Materials and methods

2.1 Participants

Participants in this study were recruited from referrals to a university hospital developmental disabilities specialty clinic located on Long Island, New York. All families with at least one child with a confirmed diagnosis of ASD were contacted by mail for participation in genetic research. A total of 92 individuals were initially recruited, but to maximize homogeneity, the study sample (N=64) was limited to individuals who were children (4–14 years old) when the diagnostic and behavioral evaluations were conducted. The study protocol stipulated that children would be excluded if a Rett MECP2 or a Fragile X mutation was discovered; however, none were found. Demographic characteristics were as follows: age (M=6.9; SD=2.6), gender (87% male), ethnicity (96% Caucasian), IQ (M=79.2; SD=23.2), socioeconomic status (SES) assessed with Hollingshead's (1975) index of occupational and educational social status (M=42.4; SD=11.4), single-parent household (1%), and psychotropic medication use (24%). DNA samples and maternal ratings were available for 59 mother-child dyads, and DNA samples were available for 53 father-child dyads. This study was approved by a university Institutional Review Board; informed consent was obtained; and appropriate measures were taken to protect patient (and rater) confidentiality.

2.2 Procedure

Diagnoses of ASD were confirmed by an expert diagnostician and based on five sources of information about ASD symptoms to verify DSM-IV criteria: (a) comprehensive developmental history, (b) clinician interview with child and caregiver(s), (c) direct observations of the child, (d) review of validated ASD rating scale data including the Child Symptom Inventory-4 (CSI-4) (De Vincent & Gadow, 2009; Gadow et al., 2008c), (e) prior evaluations, and additionally (*n*=49) with (f) the Autism Diagnostic Observation Schedule (Lord et al., 2000) and/or Autism Diagnostic Interview-Revised (Rutter et al., 2003).

Prior to scheduling their initial clinic evaluation, the parents of potential participants were mailed a packet of materials including behavior rating scales, background information questionnaire, and permission for release of school reports, psycho-educational, and special education evaluation records. Rating scales included the parent version of the CSI-4, which was completed by the child's mother. Genotype status was determined using DNA isolated from peripheral blood cells and polymerase chain reaction.

2.3 Genotyping

Polymerase chain reaction was carried out in a total volume of 20 μl with forward (5'-GCGACTACGTGGTCTACTCG -3') and reverse primers (5'-AGGACCCTCATGGCCTTG). Each amplification contained 20 ng of genomic DNA, 1 × multiplex master mix (Qiagen, Valencia, CA) and 1 μM each of the primers. Reaction conditions began with an initial denaturation at 98° C for 15 minutes, followed by 40 cycles of 98° C for 30 seconds, 60° C for 90 seconds, and 72° C for 60 seconds, with a final extension step of 10 minutes at 72° C. Products were analyzed on a QIAxcel System (Qiagen, Valencia, CA) and genotype analysis conducted by an investigator (D.O. and V.P.) who were blind to the behavioral characteristics of the study sample.

2.4 Measures

The CSI-4 (Gadow & Sprafkin 1986, 2002) is a behavior rating scale that assesses the behavioral symptoms of a broad range of psychiatric syndromes. Individual items bear one-to-one correspondence with DSM-IV symptoms (i.e., high content validity). To assess symptom severity, items are scored (never=0, sometimes=1, often=2, and very often=3) and summed separately for each disorder's symptoms. In the present study, analyses pertained to two behavioral domains: oppositionality (ODD, SAD) and repetitive behaviors (OCB, tics). The findings of numerous studies indicate that the CSI-4 demonstrates satisfactory psychometric properties in community-based normative, clinic-referred non-ASD, and ASD samples (see Gadow & Sprafkin, 2009). Moreover, confirmatory factor analysis in a large (*N*=730) sample of children with diagnosed ASD supports the construct validity of DSM-IV syndromes (Lecavalier et al., 2009).

2.5 Statistical analyses

Prior to conducting our planned analyses, dependent variables (i.e., ODD, SAD, OCB, tics) were examined for outliers, skewness, and kurtosis. Variables not normally distributed were transformed using the square root function. Covariates to be included in subsequent analyses were identified by examining potential maternal and paternal genotype group differences in demographic characteristics, as well as associations between these demographic variables and the dependent variables. Chi-square tests (categorical variables), correlations (continuous variables), and ANOVAs (combined categorical and continuous variables) were used to test these relations.

The first step in the analyses was to conduct separate MANOVAs to determine which child behaviors from the two domains of interest were associated with maternal genotype. Because evidence points to the 7-repeat allele as a potential risk variant in both children (Smith, 2010) and adults (Congdon et al., 2008; van IJzendoorn et al., 2008), we adopted a widely used procedure of comparing 7-repeat allele carriers versus non-carriers. This also reduced the number of potential genotype groups, which had important statistical advantages. We limited examination of subsequent univariate analyses to situations where the multivariate *F* was significant, thereby reducing the risk of Type 1 error for multiple, related variables.

Next we constructed four groups of mother-child dyads based on whether individuals were 7-repeat allele carriers (7+) or noncarriers (7−): MC7−, MC7+, M7+/C7−, and M7−/C7+. In order to maximize our ability to detect group differences, all *á priori* pairwise comparisons included the largest group (MC7− dyads). With these comparisons we sought to determine if (a) mother-child genotype was associated with symptom severity (MC7−<MC7+), and (b) mothers' genotype was associated with ratings of misbehavior in noncarrier offspring (MC7−<M7+/C7−). We repeated the aforementioned analyses using father-child dyads and parent-child triads to determine whether these configurations would also be more informative as biomarkers of symptom severity. There were two situations where mother, father, and child were all 7-repeat allele carriers.

We calculated partial eta-squared (ηp^2) to gauge the magnitude of group differences and to address in part the inherent limitations of significance testing (Cohen, 1994; Feise, 2002; Perneger, 1998; Rothman, 1990; Zhang et al., 1997). A rule of thumb for determining the magnitude of ηp^2 suggests the following: 0.01–0.06=small, 0.06–0.14=moderate, and >0.14 = large (Cohen, 1988).

Family-based analyses were carried out using the Transmission Disequilibrium Test (TDTae; ae=allowance for errors) program 2.0 (Gordon et al., 2001, 2004; Yang et al., 2008), which is available online (http://linkage.rockefeller.edu/pawe). TDTae allows for both Mendelian inconsistencies resulting from random genotyping errors and missing parental genotype data. Children were separated into two groups comprised of youngsters with more and less symptom severity (median split). Variables not normally distributed were transformed with the square root function. Owing to the fact that these analyses were exploratory and symptom variables were not independent, no corrections were made for multiple comparisons.

3. Results

Mothers' *DRD4* allelic frequencies were as follows: allele 2 (12%), allele 3 (2.5%), allele 4 (66%), allele 5 (2.5%), allele 6 (1%), and allele 7 (16%); fathers' allelic frequencies showed a similar distribution: allele 2 (12%), allele 3 (2%), allele 4 (64%), and allele 7 (22%). Neither mothers' nor fathers' allele distributions deviated from Hardy-Weinberg equilibrium (X^2 =0.58, p=0.45; X^2=1.71, p=.19, respectively). The distribution of mothers'/fathers' genotype groups were 7-repeat allele carriers (n=18, 31%/ n=18, 34%) and noncarriers (n=41, 69%/ n=35, 66%), respectively.

Importantly, mothers' and fathers' genotype groups did not significantly differ with regard to child's IQ or severity of the three core domains of ASD symptomatology (communication and social deficits, perseverative behaviors). Neither did the genotype groups differ in demographic characteristics (i.e., child's age, gender, ethnicity, psychotropic medication, special education, mothers' level of education, family's SES, or whether the mother was a single-parent).

3.1 Mothers' genotype

MANOVAs did not indicate multivariate effects of mothers' genotype for either ODD/SAD (F=2.72, p=0.08) or repetitive behavior (F=1.15, p=0.33); therefore, follow-up univariate analyses were not conducted for these variables.

3.2 Mother-child dyads

Mother-child dyads were as follows: MC7− (n=32), MC7+ (n= 10), M7+/C7− (n=8), and M7−/C7+ (n=9). The multivariate effect was significant for ODD/SAD (F=2.81, p=0.02) as were the univariate analyses for both ODD (F=2.83, p=0.05, ηp^2=0.15) and SAD (F=3.75, p=0.02, ηp^2=0.19). For ODD symptoms, planned comparisons indicated that the MC7− dyad had less severe symptoms than the MC7+ dyad (p=0.02, ηp^2=0.11) (Figure 1). There was no statistically significant evidence that mothers' genotype may have influenced ratings of ODD symptoms. In other words, noncarrier children were rated similarly by noncarrier (MC7−) and 7-repeat allele carrier (M7+/C7−) mothers.

For SAD (Figure 1), planned comparisons indicated that children in the MC7− dyad had less severe SAD than youngsters in the MC7+ dyad (p<0.01; ηp^2=0.19). Moreover, exploratory analyses indicated significant differences between the MC7+ dyad and the M7+/C7− (p=0.01; ηp^2=0.15) and M7−/C7+ (p=0.02; ηp^2=0.23) dyads suggesting that the MC7+ genotype configuration may be a potential biomarker for SAD severity.

The multivariate effect of mother-child dyads was not significant for repetitive behaviors (F=1.38, p=0.23); therefore, follow-up univariate analyses were not conducted.

3.3 Fathers' genotype

For analyses involving fathers' genotype, it is helpful to recall that ratings of child behavior are based on maternal report. MANOVA was not significant for ODD/SAD (F=2.14, p=0.13), but there was a significant multivariate effect for repetitive behaviors (F=5.15, p<0.01). Univariate analyses were significant for both OCB (F=5.40, p=0.03, ηp^2=0.11) and tics (F=7.36, p<0.01, ηp^2=0.14). Planned comparisons indicated that children whose fathers were 7-repeate allele carriers were rated as having more severe OCB (p=0.03, ηp^2=0.11) and tics (p<0.01, ηp^2=0.14) than youths whose fathers were noncarriers. This suggests that paternal *DRD4* genotype may be a potential biomarker for repetitive behaviors regardless of child genotype.

3.4 Father-child dyads

Father-child dyads were as follows: FC7− (n=28), FC7+ (n=11), F7+/C7− (n=7), and F7−/C7+ (n=7). Unlike the findings for mother-child dyads, the MANOVA for father-child dyads was not significant for ODD/SAD (F=1.85, p=0.10). The MANOVA was, however, significant for repetitive behaviors (F=2.95, p=0.01) as was the univariate test of tic severity (F=3.80, p=0.02, ηp^2=0.21). OCB ratings were marginally significant (F=2.92, p=0.05, ηp^2=0.17).

Children in the FC7− dyads were rated as having less severe tics than youngsters in the FC7+ (p=0.02, ηp^2=0.18) and F7+/C7− (p=0.01, ηp^2=0. 21) dyads (Figure 2). In addition, exploratory analyses also indicated the difference between the FC7− and F7−/C7+ dyads was marginally significant (p=0.07). Collectively, these results suggest that having at least one parent who is a *DRD4* 7-repeat allele carrier may be a potential biomarker for tic severity, regardless of the child's 7-repeat allele status. This hypothesis is examined in the following section.

Planned comparisons indicated children in FC7− dyads were rated as having less severe OCB than youths in FC7+ dyads (p<0.01, ηp^2=0.19) (Figure 2). Moreover, exploratory analyses indicated marginally significant differences between the FC7+ dyad and the F7+/C7− (p=0.08)

and F7−/C7+ (p=0.05) dyads suggesting that the FC7+ genotype configuration may be a potential biomarker for OCB severity.

3.5 Parent-child triads

We also compared families with the following three genotype configurations: child and at least one parent was a 7-repeat allele carrier (PC7+; n=17), child was a noncarrier but at least one parent was a 7-repeat allele carrier (P7+/C7−; n=13), and child plus both parents were noncarriers (PC7−; n=19). The MANOVA was significant for repetitive behaviors (F=2.73, p=0.03) but not ODD/SAD (F=1.94, p=0.11). Subsequent univariate analyses indicated genotype groups differed for tic (F=4.42, p=0.02, ηp^2=0.16) but not OCB (F=2.22, p=0.12, ηp^2=0.09) severity. Children in PC7− triads were rated as having less severe tics than youths in either PC7+ (p<0.01, ηp^2=0.20) or P7+/C7− (p=0.04, ηp^2=0.14) genotype groups (Figure 3).

3.6 Allelic transmission

TDTae analyses indicated that estimated genotyping error rates were uniformly zero (i.e., no instance of parent-child incompatibility). When the sample was dichotomized into more and less severe groups using a median split procedure, analyses indicated undertransmission of the 2 allele in the group of youths with more severe tics (X^2=8.25, p=0.02), which was still significant when corrected for multiple comparisons (p=0.03). For OCB, there was marginally significant evidence for the undertransmission of the 7 allele (X^2=5.78, p=0.06).

4. Discussion

The results of this study suggest that knowledge of parental genotype, in conjunction with child genotype, may be helpful in identifying prognostic biomarkers for co-occurring behavioral disturbances in children with ASD. Although optimal *DRD4* genotype configuration varied as a function of parent (mother, father) and symptom dimension, obtained effect sizes for particular subgroup comparisons were generally larger than for gene-behavior associations based solely on child genotype. Specifically, mother-child and father-child 7-repeat genotypes were associated with ODD and SAD severity and with OCB and tic severity, respectively. In addition, youths who did not have a parent who was a 7-repeat carrier (which comprised 39% of the study sample) were less likely to have severe tic-like behaviors than the 61% who did (i.e., regardless of the child's genotype), and the magnitude of this group difference was large (p=0.005, ηp^2=0.16). Nevertheless, effect sizes for tic severity were even larger for some subgroup comparisons of father-child dyads. Because there are seemingly countless biopsychosocial variables that contribute to variation in parent and child behavior, to include gene-gene and gene × environment interactions, parent-of-origin effects, and allelic and locus heterogeneity, the search for clinically useful prognostic biomarkers is a dynamic process that will need to accommodate to ever more complex models of disease and prevention, requiring ever larger samples. Moreover, this diversity in sources of variation and mechanisms of pathogenesis may also explain the less than perfect alignment with specific findings for allelic transmission.

Studies examining the relation of parental genotype with variables associated with child behavior problems are limited, and all pertain to non-ASD samples. In one study of toddlers at risk for disruptive-aggressive behavior problems, van IJzendoorn et al. (2008) compared mothers who were and were not carriers of genes associated with less efficient dopaminergic system functioning (i.e., *DRD4* 7-repeat allele carriers, *COMT* Val+ genotype). Mothers with a combination of both "risk" genotypes engaged in less than optimal parenting behavior if they were also confronted with higher levels of daily hassles versus similar parents with lower levels of daily hassles. Moreover, the latter were actually more sensitive parents than the comparison

genotype groups. Gervai et al. (2007), however, failed to detect an association between maternal *DRD4* genotype and level of disrupted maternal affective communication with infants, but a recently reported study by Kaitz et al. (2010) found that mothers who were *DRD4* 7-repeat carriers behaved more sensitively toward fussy than less fussy infants compared with mothers who were not 7-repeat allele carriers. Lastly, Lee et al. (2008) reported an association between a maternal dopamine transporter gene (*DAT1*, *SL6A3*) polymorphism and negative parenting in a sample of children with ADHD and controls, which was significantly stronger for children who were highly disruptive during a mother-child interaction task.

Given the (a) well-documented stress involved in raising a child with ASD and (b) evidence in adults associating the 7-repeat allele with impulsivity (Congdon et al., 2008) and less than optimal parenting in stressful situations (van IJzendoorn et al., 2008)), we explored whether mothers' *DRD4* genotype would influence ratings of symptom severity, either directly through reciprocal interaction with the child or indirectly though altered perceptions of misbehavior. Although noncarrier children in our study were not rated more severely by mothers who were carriers versus noncarriers, mean ratings for ODD and SAD symptom severity were in the predicted direction (Figure 1). However, the modest size of the study sample and concerns about Type 2 error restrict inferences to suggestive evidence. Conversely, it is possible that susceptibility alleles are differentially more problematic for children with certain types of central nervous system disease than for their parents (who do not have the disorder). Alternatively, if 7-repeat allele carriers do engage in less effective parenting (which was not assessed in this study), their noncarrier offspring may be buffered from the experience by the behavior of a noncarrier spouse or partner. Regardless, further study with larger samples is warranted.

We have found associations of repetitive behaviors with both impulsive-disruptive behaviors and anxiety in this same sample for other candidate genes that influence dopaminergic system function or development (Gadow et al., 2008b, 2009; Roohi et al., 2009) as have others for non-ASD samples (Comings et al., 1996; Rowe et al., 1998). This is consistent with research findings from diverse disciplines to include the following: (a) co-occurrence of these behaviors in animals (Hutt & Hutt, 1965; Koolhaas et al., 1999; Sih et al., 2004) and many different neurodevelopmental syndromes in humans, (b) phylogeny of seemingly purposeless movements in emotional expression of animals and humans (Darwin, 1890, Sherrington, 1900) and their phenomenological similarities (Eilam et al., 2006; Tinbergen & Tinbergen, 1976), (c) both phenotypic and genotypic evidence of routines or repetitive behaviors in proactive animal personality (Koolhaas et al., 1999; Sih et al., 2004), which may include high levels of exploratory behavior, activity, and aggression but low levels of flexibility, arguably a characteristic of ODD and SAD, (e) likely role of repetitive behaviors in anxiety/stress reduction (Eilam et al., 2006; Hutt & Hutt, 1965; Koolhaas et al., 1999), and (f) involvement of the dopaminergic system in repetitive behaviors in animals (Eilam et al., 2006; Koolhaas et al., 1999) and humans (see below). Based on the aforementioned research, it also seems reasonable to speculate that for some children with ASD, repetitive behaviors may compensate for a seemingly diminished capacity for exploratory behavior (Hutt, 1969; Tinbergen & Tinbergen, 1976). Although evidence supporting the heritability of certain types of parenting behaviors is compelling (McGuire, 2003) and findings supporting an association of the 7-repeat DRD4 allele with child and parent behaviors is growing, the biologic substrates of these gene-behavior relations, both individually and interactively, are largely unknown, but progress is being made in this area as well (see McCormack et al., 2009; Meaney & Szyf, 2005; Swain et al., 2007).

Because children with ASD have seemingly high rates of OCB, tics, and SAD (e.g., Gadow & DeVincent, 2005; Gadow et al., 2005; Zandt et al., 2007) as do their relatives (Bolton et al., 1998; Micali et al., 2004; Piven & Palmer, 1999), it is possible *DRD4* exon 3 VNTR variants

may actually be implicated in the pathogenesis of behavioral disturbances. In the case of repetitive behaviors, TDTae analyses indicated that greater versus lesser tic severity (categorical model) was associated with the 2-repeat allele (undertransmission) with marginally significant evidence for the 7-repeat allele (undertransmission) and OCB. The relation of *DRD4* alleles with obsessive-compulsive disorder, tic disorder, or both in non-ASD samples has been examined with both case-control and within-family transmission analyses in studies from North America (Billett et al., 1998; Camarena et al., 2007; Cruz et al., 1997; Díaz-Anzaldúa et al., 2004; Grice et al., 1996), Europe (Millet et al., 2003; Tarnock et al., 2007; Walitzia et al., 2008), Isreal (Frisch et al., 2000) and South Africa (Hemmings et al., 2004). Of the studies that examined tics, several provide at least tentative evidence supporting an association with *DRD4* (Billet et al., 1998; Camarena et al., 2007; Cruz et al., 1997; Díaz-Anzaldúa et al., 2004; Grice et al., 1996; Walitzia et al., 2008), but results vary as a function of methodology, risk/protective allele, and patient characteristics, and at least two studies were negative (Millet et al., 2003; Tarnock et al., 2007). Although linkage disequilibrium may explain some discrepancies (Lin et al., 2007), replication drift, especially in view of the diversity in assessment strategies for measuring the phenotype, is also a concern as are trait heterogeneity and gene × environment interactions (Moffitt et al., 2005) to name but a few. For these and other reasons it is nevertheless remarkable that this particular locus has been implicated in so many different studies.

4.1 Limitations and directions for future research

Our results are subject to at least several qualifications. We used a dimensional strategy to measure the severity of co-occurring symptoms in children with diagnosed ASD as compared with categorical psychiatric diagnoses, so obtained findings may not apply to the latter. The modest size of the study sample increases the probability of spurious findings, decreases our ability to detect valid gene-behavior associations, and prevents more detailed analyses of parent-of-origin effects. It also precludes comparison of various combinations of parent-child genotypes to include the 2-repeat allele, which may be functionally intermediate between the 4- and 7-repeat alleles (Armbruster et al., 2009; Kang et al., 2008; Wang et al., 2004), and heterozygotes (Comings & MacMurray, 2000). Other variables of concern in terms of informing etiology include referral bias, linkage disequilibrium, and in the case of the MANOVA analyses, population structure (Cardon & Palmer, 2003), although meta-analyses of gene-disease research (Bamshad, 2005; Goldstein & Hirschorn, 2004; Ioannidis et al., 2004) and review of the extant literature (Hutchison et al., 2004) suggest this potential threat to internal validity may be overstated. Moreover, in the present study, because controls were ASD children with less severe symptoms from the same primarily Caucasian sample and were recruited and genotyped in identical fashion and at the same time, it is less likely that population structure or genotyping error confounded obtained results. Nevertheless, this remains a possibility. Lastly, we conceptualized the 7-repeat allele as a “risk genotype” when in fact interactions with favorable environmental experiences may facilitate more desirable outcome for some children or their parents (Bakermans-Kranenburg & van IJzendoorn, 2007; Belsky et al., 2009; van IJzendoorn et al., 2008), which if true may have led to the under-representation of 7+/7+ dyads with exceptionally non-conflicted interactions in our sample.

We did not investigate whether parental 7-repeat allele was actually associated with differentially higher levels of stress or less effective parenting, both of which may be important in explaining the observed association between parents’ *DRD4* genotype and child behavior (e.g., Gervai et al., 2007; van IJzendoorn et al., 2008). The particular pattern of behaviors (ODD, SAD) associated with *DRD4* genotype share similarities with disorganized attachment, which may play a role in the pathogenesis of disruptive behavior disorder (see Kochanska et al., 2009). Because polygeny and epistasis are part of the genetic architecture of behavioral characteristics (Flint & Mackay, 2009; Moore, 2003), it is reasonable to expect that multiple

genes are involved in parenting behavior as well. For example, there is another variation in the *DRD4* gene, a single nucleotide polymorphism, -521 C/T, also shown to reduce transciptional activity (Okuyama et al., 1999) that may be associated with mother-child interactions (see Bakermans-Kranenburg & van Ijzendoorn, 2007).

Although the present study obtained fairly detailed accounts of child behavior problems, this was not the case for the mental health status of the children's mothers or fathers. For example, there is some indication that ODD is a viable clinical phenotype in adults (Gadow et al., 2007), and it would be informative to determine if parents' *DRD4* genotype is associated with behavioral characteristics that might influence parenting. Moreover, there is some research indicating that child misbehavior appears to have relatively less significance for paternal than maternal stress (e.g., Hastings et al., 2005; Herring et al., 2006), possibly as a function of differentially less involvement in child care, which also warrants closer examination.

For all the aforementioned reasons, our reported findings must be considered tentative pending replication in larger independent samples. Moreover, they are hypothesis generating and not hypothesis confirming and as such are presented here as indications for further study in what has to date been a relatively ignored topic within the ASD clinical phenotype.

4.2 Clinical implications

Co-occurring behavioral disturbances in children with ASD pose serious challenges to intervention efforts, function as critical impediments to social integration with peers and later life adjustment; and typically result in considerable stress in the home. The identification and validation of potential prognostic biomarkers is a necessary first step in the formulation of genomic profiles that can be ascertained at the point of diagnosis and later used to inform treatment and long-term clinical management decisions. The findings of the present study suggest one possible strategy for enhancing the predictive power of common gene variants as possible biomarkers of behavioral disturbances in children with ASD and indicate directions for further study.

Abbreviations

ADHD	attention-deficit hyperactivity disorder
ASD	autism spectrum disorder
CSI-4	Child Symptom Inventory-4
DAT1	dopamine transporter gene
DRD4	dopamine receptor D4 gene
ηp^2	partial eta-squared
F	father
FC	father-child
M	mother
MC	mother-child
OCB	obsessive-compulsive behavior
ODD	oppositional defiant disorder
SAD	separation anxiety disorder
SES	socioeconomic status

TDT	transmission disequilibrium test
VNTR	variable number tandem repeat

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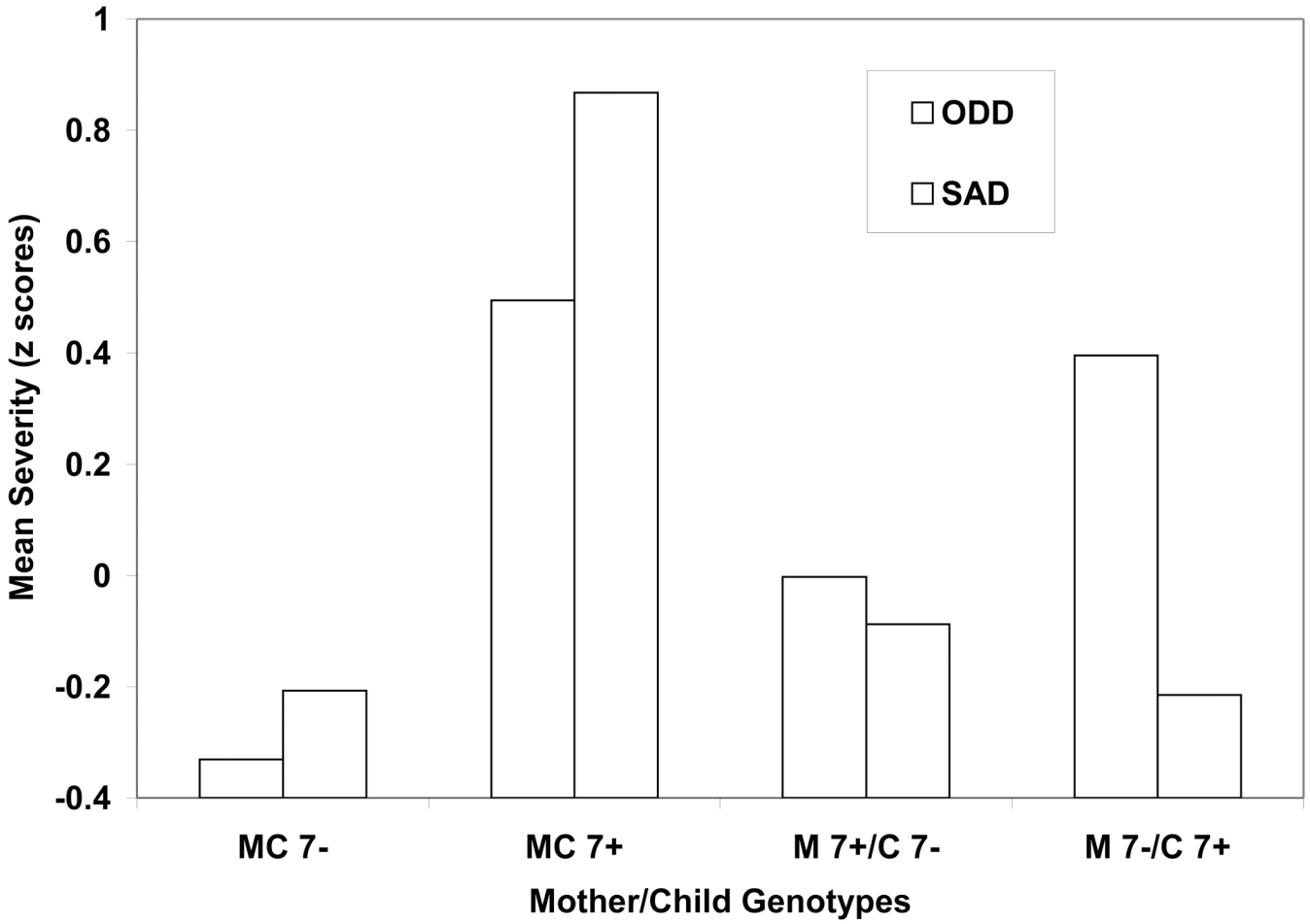

Figure 1.
Mother-child dyads (MC), *DRD4* 7-repeat allele carriers (7+) and noncarriers (7−), and maternal ratings of oppositional defiant disorder (ODD) and separation anxiety disorder (SAD) symptom severity (*z* scores).

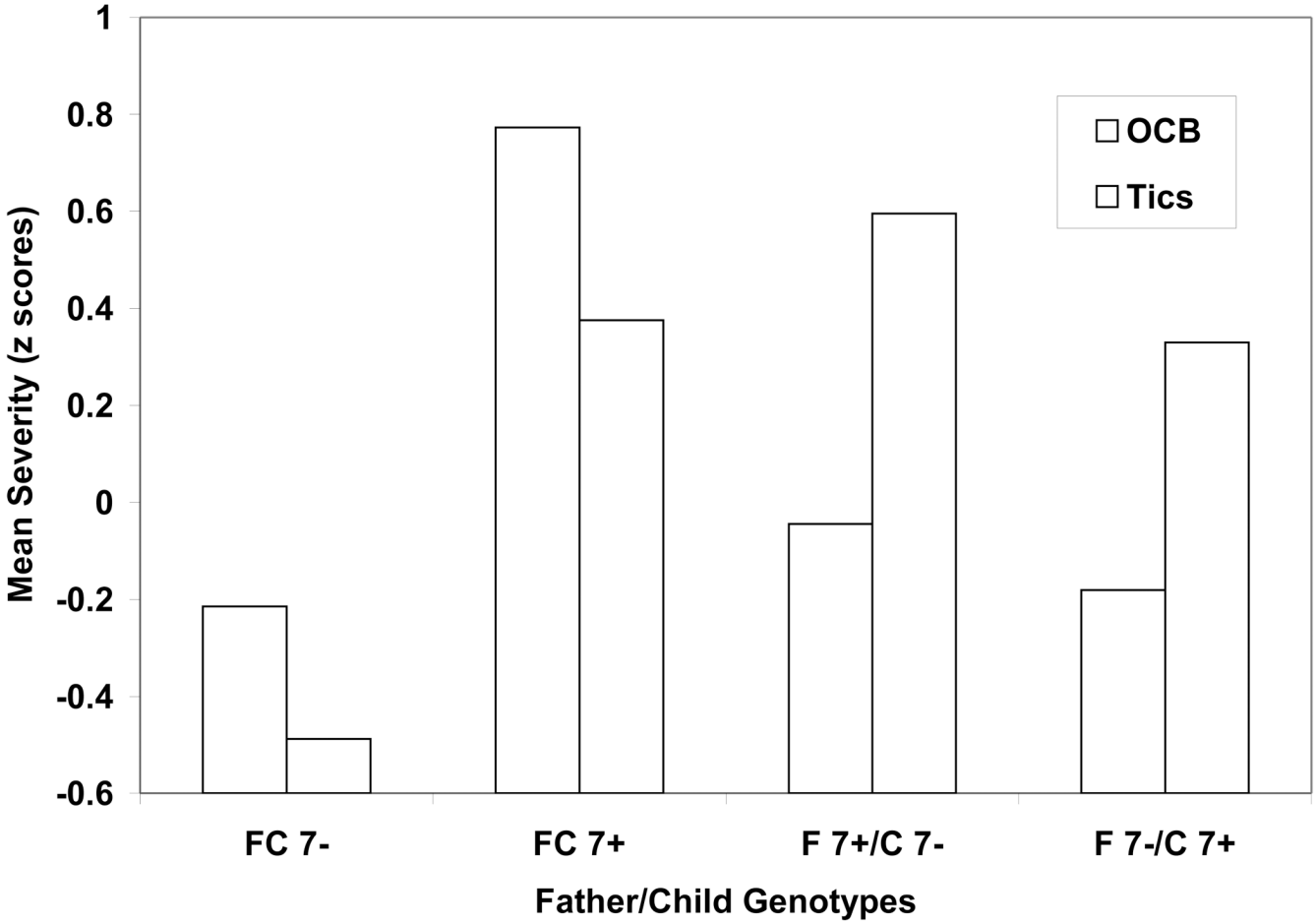

Figure 2.
Father-child dyads (FC), *DRD4* 7-repeat allele carriers (7+) and noncarriers (7−), and maternal ratings of obsessive-compulsive behavior (OCB) and tic severity (*z* scores).

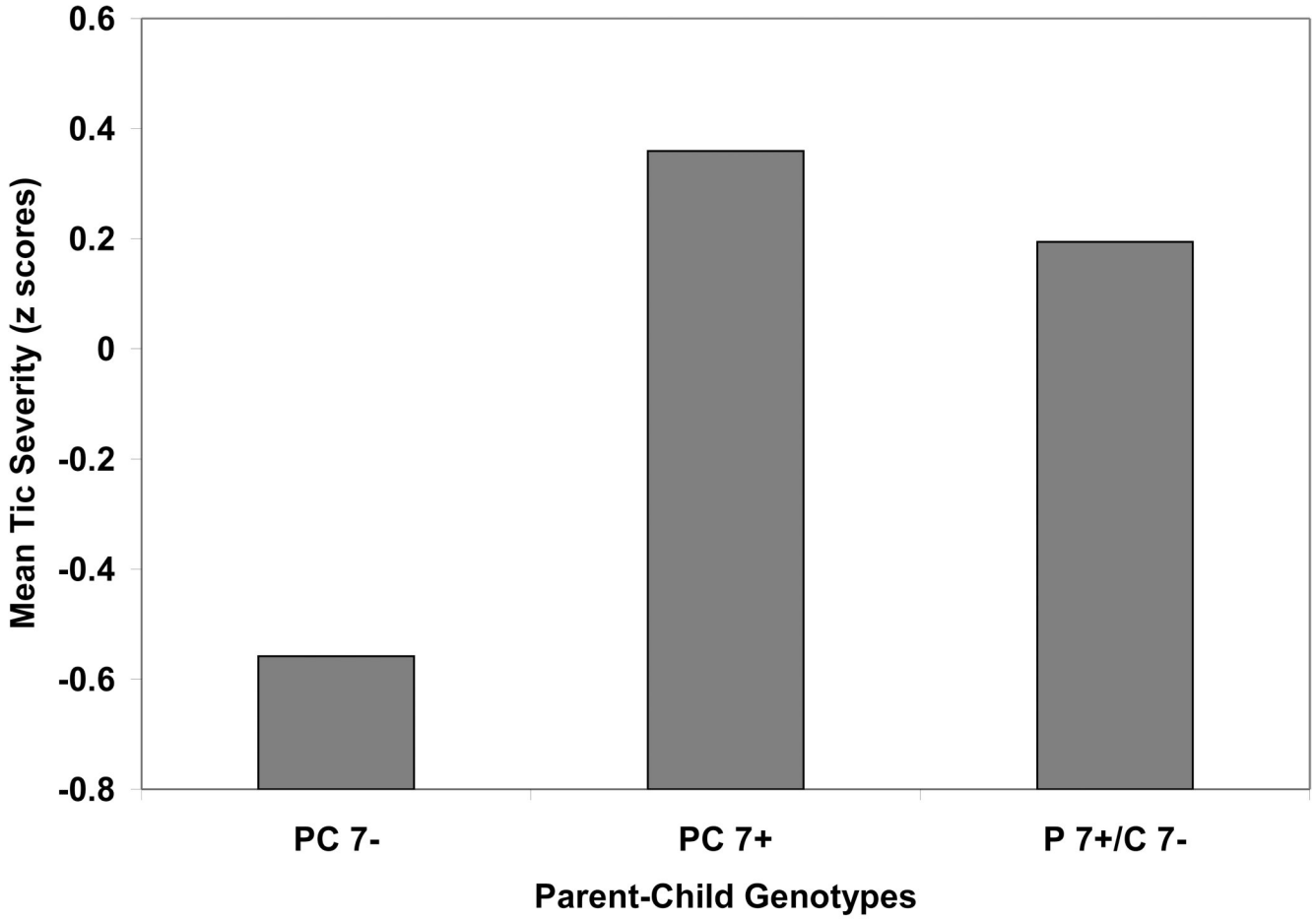

Figure 3.
Parent-child triads (PC), *DRD4* 7-repeat allele carriers (7+) and noncarriers (7−) and maternal ratings of tic severity (*z* scores).