Dopaminergic Variants in Siblings at High Risk for Autism: Associations With Initiating Joint Attention

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Younger siblings of children with autism spectrum disorder (ASD; high-risk siblings) exhibit lower levels of initiating joint attention (IJA; sharing an object or experience with a social partner through gaze and/or gesture) than low-risk siblings of children without ASD. However, high-risk siblings also exhibit substantial variability in this domain. The neurotransmitter dopamine is linked to brain areas associated with reward, motivation, and attention, and common dopaminergic variants have been associated with attention difficulties. We examined whether these common dopaminergic variants, *DRD4* and *DRD2*, explain variability in IJA in high-risk ($n = 55$) and low-risk ($n = 38$) siblings. IJA was assessed in the first year during a semi-structured interaction with an examiner. *DRD4* and *DRD2* genotypes were coded according to associated dopaminergic functioning to create a gene score, with higher scores indicating more genotypes associated with less efficient dopaminergic functioning. Higher dopamine gene scores (indicative of less efficient dopaminergic functioning) were associated with lower levels of IJA in the first year for high-risk siblings, while the opposite pattern emerged in low-risk siblings. Findings suggest differential susceptibility—IJA was differentially associated with dopaminergic functioning depending on familial ASD risk. Understanding genes linked to ASD-relevant behaviors in high-risk siblings will aid in early identification of children at greatest risk for difficulties in these behavioral domains, facilitating targeted prevention and intervention. ***Autism Res*** *2016, 00: 000–000*. © 2016 International Society for Autism Research, Wiley Periodicals, Inc.

Keywords: high-risk siblings; initiating joint attention; dopamine; differential susceptibility; autism spectrum disorder

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by a broad range of social and communication impairments and stereotyped patterns of behavior [American Psychiatric Association, 2013], with prevalence estimates of over 1 in 75 children [CDC, 2014]. The younger siblings of children with ASD (high-risk siblings) have high rates of ASD diagnosis, with recurrence rates of 4.5–18.7%, and exhibit substantial heterogeneity in behaviors associated with ASD [Grønborg, Schendel, & Parner, 2013; Messinger et al., 2013; Ozonoff et al., 2011; Risch et al., 2014], including initiating joint attention (IJA). Common genetic variants such as those seen in dopaminergic genes *DRD4* and *DRD2* may aid in understanding the variability of phenotypic presentation in high-risk siblings. The current study examined these dopaminergic variants in high-risk siblings and low-risk siblings (siblings with no family history of ASD) to better understand heterogeneity in a behavioral phenotype particularly relevant to ASD, initiating joint attention.

Genetics and ASD

Although recent estimates suggest substantial heritability for ASD [Colvert et al., 2015; Hallmayer et al., 2011], specific genes responsible for this heritability are not always clear [Geschwind, 2011]. Both rare and common variants contribute to understanding genetic susceptibility in ASD. Several rare variants (mutations with a minor allele frequency of less than 1%) associated with ASD have been identified [Betancur, 2011; Buxbaum, 2009; Geschwind & State, 2015]. Gene discovery efforts have yielded a number of ASD susceptibility genes, such as *CHD8*, *CNTNAP2*, *NLGN4*, *NRXN1*, and *CNTN4* [e.g., Chen, Peñagarikano, Belgard, Swarup, & Geschwind, 2015; De Rubeis et al., 2014; Iossifov et al., 2014]. However, no specific gene accounts for a majority of ASD cases [Abrahams & Geschwind, 2008; Geschwind, 2011; Muhle, Trentacoste, & Rapin, 2004]. Even among siblings both diagnosed with ASD, most do not share the same ASD risk genes, underscoring the genetic heterogeneity of ASD [Yuen et al., 2015] and highlighting the potential difficulty of identifying replicable ASD

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susceptibility genes. Common variants (polymorphisms that occur in greater than 1–2% of the population) may comprise a substantial portion of the risk heritability of ASD [Gaugler et al., 2014; Klei et al., 2012]. However, identified common variants that in combination or alone influence ASD susceptibility have not been well-replicated [Anney et al., 2010; Devlin, Melhem, & Roeder, 2011; Muhle et al., 2004]. As genetic underpinnings of ASD are highly heterogeneous and a number of genes likely interact to influence susceptibility [Talkowski, Minikel, & Gusella, 2014], an approach focusing on the genetic basis of behaviors relevant to ASD may be productive in identifying genotypes associated with specific ASD-related traits [Muhle et al., 2004].

Heterogeneity Within ASD

In addition to ASD's genetic variability, ASD is phenotypically heterogeneous, encompassing a broad spectrum of impairment. Those diagnosed can exhibit varied combinations of traits and symptoms [Rapin, 1991; Rutter & Schopler, 1987], resulting in a range of later outcomes [Howlin, Goode, Hutton, & Rutter, 2004]. ASD-relevant behaviors, which are characteristic of the disorder and its symptomatology, show substantial variability in both children with ASD and in their younger siblings. Even without an ASD diagnosis, high-risk siblings exhibit elevated ASD symptoms, lower levels of developmental functioning, and behavioral difficulties [Gangi, Ibañez, & Messinger, 2014; Georgiades et al., 2013; Messinger et al., 2013].

In low-risk children, common genetic variants have been linked to behavioral phenotypes [e.g., Bakermans-Kranenburg & van IJzendoorn, 2011; Lackner, Sabbagh, Hallinan, Liu, & Holden, 2012; Posner, Rothbart, & Sheese, 2007]. Here, we aimed to examine common genetic variants implicated in behavior in the context of familial risk for ASD, to determine whether these variants may play a role in the heterogeneity seen in behaviors that are relevant to and have implications for ASD. Though individual common genetic variants are unlikely to distinguish children with ASD from case controls, these variants may be related to phenotypic variability in ASD-relevant behaviors [Geschwind, 2011] among high-risk siblings. We examined the role of two common genetic variants (*DRD4* and *DRD2*) in a sample including high-risk siblings to understand phenotypic, behavioral heterogeneity in the context of familial ASD risk.

Dopaminergic Variants and Behavior

While relationships between dopaminergic variants and behavior have been studied in typically developing children, there has been little examination in children at risk for ASD. Dopamine is a catecholamine that functions as a neurotransmitter in the brain, and it plays a role in several key domains including attention, reward-motivated behavior, and motor control. Dopamine is produced in brain areas including the substantia nigra and ventral tegmental area and then is transmitted through several main pathways, some of which are associated with the control of motivation-linked systems relevant to the current study. Specifically, the mesolimbic and mesocortical pathways begin in the ventral tegmental area and connect to the nucleus accumbens and cerebral cortex, respectively, and they are associated with response to reward and motivation.

Several common polymorphisms affect dopamine neurotransmission. The *DRD4* gene encodes for dopamine receptor D4, which is expressed in areas including the frontal cortex, hippocampus, amygdala, and hypothalamus [Beaulieu & Gainetdinov, 2011]. Variants in a 48-base pair variable number tandem repeat of *DRD4* can influence gene expression, and a "long" version (the 7-repeat allele) has been associated with suppressed receptor expression [Schoots & Van Tol, 2003]. The 7-repeat allele has been associated with varied attentional and behavioral difficulties in typically developing children and infants [Auerbach, Benjamin, Faroy, Geller, & Ebstein, 2001a, Auerbach, Faroy, Ebstein, Kahana, & Levine, 2001b; Gizer, Ficks, & Waldman, 2009; Schmidt, Fox, Perez-Edgar, Hu, & Hamer, 2001]. The *DRD2* gene encodes for the dopamine receptor D2, which is expressed in areas including the striatum and nucleus accumbens [Beaulieu & Gainetdinov, 2011], and is associated with the Taq1A polymorphism on *ANKK1*. The A allele of the polymorphism (hereafter *DRD2*) is linked to a reduction in D2 receptor expression [Thompson et al., 1997] and is associated with risk for ASD and social interaction and communication difficulties [Hettinger et al., 2012; Salem et al., 2013].

ASD-Relevant Behavior

We focused on a specific ASD-relevant behavior, initiation of joint attention. Early deficits in initiating joint attention (IJA), a form of referential communication involving the use of gaze and gesture to coordinate attention between social partners and objects, are a core feature of ASD [Dawson et al., 2004; Mundy, Sigman, Ungerer, & Sherman, 1986]. Among high-risk siblings, early IJA is predictive of later ASD symptomatology [Ibañez, Grantz, & Messinger, 2012]. While some evidence suggests high-risk siblings tend to display fewer IJA behaviors than low-risk siblings [Cassel et al., 2007; Goldberg et al., 2005; Ibañez et al., 2012; Rozga et al., 2011], other investigations do not report differences [Toth, Dawson, Meltzoff, Greenson, & Fein, 2007; Yirmiya et al., 2006]. These mixed findings highlight the necessity for empirical work to explain phenotypic variability among high-risk siblings.

IJA is particularly relevant to high-risk siblings, as it may be predictive of later symptomatology. Given the role of the dopaminergic system in reward sensitivity and motivation, it may influence whether an infant finds social interaction through joint attention rewarding and is motivated to perform IJA behaviors. In addition, the dopaminergic system's role in motor control and attention may play a role in an infant's ability to shift attentional focus and execute such behaviors. Despite the relationship between dopaminergic variants and related functioning in typical development, similar associations have not been examined within children at risk for ASD. Investigating relations between behavioral phenotypes and dopaminergic genotypes in the context of familial risk for ASD may aid in understanding the manifestation of early ASD-relevant behaviors, enabling early identification of behavioral targets for early intervention.

Current Study

The current study examined dopaminergic genotypes *DRD4* and *DRD2* in relation to an ASD-relevant behavioral phenotype, initiating joint attention, in the context of familial autism risk.

Aim 1. Characterize dopaminergic genotype distributions in high-risk and low-risk siblings. We examined distributions of genotype frequencies (*DRD4*, *DRD2*, and a dopamine gene score comprised of both genes) in high- and low-risk siblings. Due to the genetic heterogeneity of ASD and little evidence that dopaminergic genes confer ASD susceptibility, we did not expect genotype frequencies to differ between groups (i.e., risk alleles would not be overrepresented in high-risk siblings).

Aim 2. Examine the relationship between dopaminergic variants and ASD-relevant behavioral phenotype in high-risk and low-risk siblings. Regression models tested the effect of dopaminergic genotype, as well as its interaction with risk group status, on IJA in the first year. Due to the potential role of dopamine in areas relevant to IJA (attentional control, social motivation and reward, motor control), we expected less efficient dopaminergic functioning to be associated with lower levels of IJA.

Methods

Participants

Participants were the infant siblings of children diagnosed with autism spectrum disorder (ASD; high-risk siblings, $n = 55$, 35 male) or the infant siblings of typically developing children with no history of ASD (low-risk siblings, $n = 38$, 16 male). High-risk siblings have at least one older sibling with a diagnosis of ASD, confirmed upon study enrollment by administration of the Autism Diagnostic Observation Schedule (ADOS) [Lord et al., 2000] and clinical diagnosis by a licensed clinical psychologist. Low-risk siblings have older siblings with no evidence of ASD, confirmed by a score lower than 9 on the Social Communication Questionnaire [Berument, Rutter, Lord, Pickles, & Bailey, 1999], a conservative cutoff score, and no family history of ASD in first degree relatives. All procedures were reviewed and approved by the University of Miami Institutional Review Board. Written informed consent was obtained from parents of all participants included in the study.

Measures

Early Social Communication Scales (ESCS). Joint attention was assessed within the ESCS [Mundy et al., 2003] at 8, 10, and 12 months. The ESCS is a semi-structured assessment of infants' nonverbal communication abilities, during which an examiner (seated across from the infant) presents and activates a series of toys, creating opportunities for the infant to initiate joint attention. After presenting and activating a toy, the examiner remains attentive and responds to the infant's joint attention bids briefly. The current study focused on initiating joint attention (IJA) bids occurring during the ESCS (e.g., when infant gazed between the examiner and activated toy or showed an object to the examiner). Videotaped assessments were reliably coded by trained coders. Rates per minute of joint attention were calculated for each assessment age; a mean was calculated from the standardized values of each assessment age to provide a measure of IJA in the first year for analyses.

Dopamine genotypes. Genetic data was collected from saliva samples from participants using Oragene DNA collection kits. Genetic samples were sent for extraction and analysis to the John P. Hussman Institute for Human Genomics (HIHG) at the University of Miami Miller School of Medicine, where genotyping was conducted for *DRD4* and *DRD2*. For analyses, genotypes for *DRD4* (rs1805186) were grouped according to the presence or absence of the 7-repeat allele ("0" = no 7-repeat, "1" = at least one 7-repeat). For *DRD2* (rs1800497), genotypes were grouped according the presence of the A allele ("0" = no A allele, "1" = at least one A allele).

A dopamine gene score was also created by coding *DRD4* and *DRD2* to reflect dopaminergic functioning. Higher scores indicated more "risk" genotypes (indexing less efficient dopaminergic functioning) and lower scores indicated fewer "risk" genotypes (indexing more efficient dopaminergic functioning). Gene scores served

as an index of cumulative dopaminergic functioning for analyses of outcomes (for similar approaches, see Nikolova, Ferrell, Manuck, & Hariri [2011], Pearson-Fuhrhop et al. [2014], and Stice, Yokum, Burger, Epstein, & Smolen [2012]), with participants coded as having 0, 1, or 2 risk genotype sets.

Analytic Approach

For Aim 1, initial analyses examined distributions of genotype frequencies, testing whether allelic frequencies were consistent with Hardy–Weinberg equilibrium. Fisher's exact tests determined whether genotype frequencies differed between high- and low-risk siblings or between ethnicities. For Aim 2, to first understand the roles of the *DRD4* and *DRD2* genotypes individually, we determined whether individual genotypes interacted with participants' risk status to predict behavioral phenotype (IJA). A 2 (genotype) by 2 (risk group) ANOVA for each of the dopaminergic variants tested for main effects of genotype and risk status, as well as their interaction. To understand the effect of dopaminergic functioning across the two genotypes, this was followed by a regression in which dopamine score, status, and dopamine score*status interaction were entered as predictors to determine the cumulative effect of both dopaminergic genes. Interaction effects were followed up with individual models by risk group in which IJA was regressed on gene score.

Results

Dopaminergic Genotypes

Allelic distributions for *DRD4*, $\chi^2(1) = 0.02$, $P = 0.85$, and *DRD2*, $\chi^2(1) = 0.00$, $P = 0.82$, were consistent with Hardy–Weinberg equilibrium [Rodriguez, Gaunt, & Day, 2009]. Allele frequencies for *DRD4*, $P = 0.82$, and *DRD2*, $P = 0.25$, did not differ between high-risk and low-risk siblings (see Table 1). Allele frequencies for *DRD4*, $P = 0.15$, and *DRD2*, $P = 0.14$, did not differ by ethnicity (coded Non-Hispanic White/Caucasian, Hispanic/Latino, and Other; see supporting information Table 1 for genotypes by ethnicity).

For analyses, genotypes for *DRD4* and *DRD2* were grouped according to the presence or absence of any alleles indicating less efficient dopaminergic functioning (7-repeat or A allele, respectively). Genotype frequencies for *DRD4*, $P = 0.48$, and *DRD2*, $P = 0.37$, did not differ between high-risk and low-risk siblings (see Table 2). Genotype frequencies for *DRD4*, $P = 0.13$, and *DRD2*, $P = 0.14$, did not differ by ethnicity. Dopamine composite scores also did not differ between high-risk and low-risk siblings, $P = 0.69$ (see Table 2), or by ethnicity, $P = 0.23$.

Table 1. DRD4 and DRD2 Allele Frequencies by Risk Group

	High-risk siblings		Low-risk siblings	
	Frequency	Percentage	Frequency	Percentage
DRD4				
–	41	74.5%	24	66.7%
7/–	13	23.6%	11	30.6%
7/7	1	1.8%	1	2.8%
DRD2				
G/G	35	63.6%	28	73.7%
A/G	19	34.5%	8	21.1%
A/A	1	1.8%	2	5.3%

Table 2. Genotype Frequencies by Risk Group

	High-risk siblings		Low-risk siblings	
	Frequency	Percentage	Frequency	Percentage
DRD4				
7-repeat allele	14	25.5%	12	33.3%
No 7-repeat allele	41	74.5%	24	66.7%
DRD2				
A allele	20	36.4%	10	26.3%
No A allele	35	63.6%	28	73.7%
Dopamine score				
0 risk genotypes	28	51.9%	19	52.8%
1 risk genotype	18	33.3%	14	38.9%
2 risk genotypes	8	14.8%	3	8.3%

Initiating Joint Attention

Preliminary analyses. Raw IJA scores (rates per minute) were examined using hierarchical linear modeling to determine whether there was a developmental change in IJA between 8 and 12 months (raw IJA scores by group are provided in supporting information Table 2). There were linear, $\beta = 0.72$, $t(89) = 4.09$, $P < 0.001$, and quadratic, $\beta = -0.35$, $t(89) = -4.23$, $P < 0.001$, effects of age. While high-risk and low-risk siblings differed in initial levels of IJA, $\beta = -0.32$, $t(88) = -2.18$, $P = 0.032$, they did not differ in their trajectories—high-risk siblings exhibited consistently lower levels of IJA than low-risk siblings from 8 to 12 months. For the purposes of subsequent analyses for this study, IJA scores were standardized at each age, and a mean was taken of these standardized scores for each infant in order to provide a measure of IJA in the first year.

DRD4. There was no main effect of genotype, $F(1, 86) = 0.05$, $P = 0.82$, partial $\eta^2 = 0.001$, on IJA. There was a main effect of group status, $F(1, 86) = 12.08$, $P = 0.001$, partial $\eta^2 = 0.12$, with high-risk siblings exhibiting lower levels of IJA than low-risk siblings. This main effect was modified by a genotype*status interaction effect, $F(1, 86) = 8.80$, $P = 0.004$, partial $\eta^2 = 0.09$ (see supporting information Figure 1). Among children without the 7-repeat allele, levels of IJA did

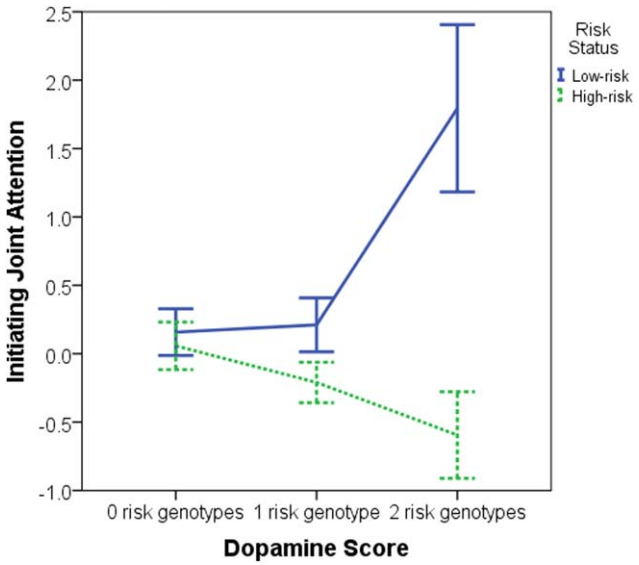

Figure 1. Mean levels of initiating joint attention (IJA) by group. Error bars reflect ± 1 SE. Initiating joint attention reflects a mean of standardized values. In high-risk siblings, 28 had a dopamine score of 0, 18 had a score of 1, and 8 had a score of 2. In low-risk siblings, 19 had a dopamine score of 0, 14 had a score of 1, and 3 had a score of 2.

not differ between high-risk ($M = 0.01$, $SD = 0.84$) and low-risk ($M = 0.11$, $SD = 0.73$) siblings, $t(62) = 0.48$, $P = 0.64$. Among children with the 7-repeat allele, however, high-risk siblings ($M = -0.52$, $SD = 0.75$) exhibited lower levels of IJA than low-risk siblings ($M = 0.73$, $SD = 1.01$), $t(24) = 3.62$, $P = 0.001$.

DRD2. There was no main effect of genotype, $F(1, 89) = 0.01$, $P = 0.91$, partial $\eta^2 = 0.00$, on IJA. There was a main effect of group status, $F(1, 89) = 6.58$, $P = 0.01$, partial $\eta^2 = 0.07$, with high-risk siblings ($M = -0.13$, $SD = 0.84$) exhibiting lower levels of IJA than low-risk siblings ($M = 0.29$, $SD = 0.86$). There was no genotype*status interaction effect, $F(1, 89) = 1.56$, $P = 0.22$, partial $\eta^2 = 0.02$ (see supporting information Figure 2).

Dopamine score. A regression model assessed effects of the dopamine score, risk group status, and their interaction on IJA, adjusted $R^2 = 0.13$, $F(3, 86) = 5.31$, $P = 0.002$. There was no main effect of status, $b = 0.03$, $t = 0.13$, $P = 0.90$. There was a main effect of dopamine score, $b = 0.50$, $t = 2.34$, $P = 0.02$, such that children with higher dopamine scores tended to have higher IJA levels. There was also a dopamine score*status interaction effect, $b = -0.81$, $t = -3.09$, $P = 0.003$. Regression analyses by risk group indicated that in high-risk siblings, IJA levels decreased as dopamine scores increased (indicative of less efficient dopaminergic functioning), $b = -0.31$, $t = -2.03$, $P = 0.047$, while in low-risk siblings, IJA levels increased as dopamine scores increased, $b = 0.50$, $t = 2.35$, $P = 0.03$ (see Figure 1). Dopamine scores explained a significant proportion of variance in IJA in high-risk siblings, adjusted $R^2 = 0.06$, $F(1, 52) = 4.13$, $P = 0.047$, and in low-risk siblings, adjusted $R^2 = 0.12$, $F(1, 34) = 5.53$, $P = 0.03$.

Discussion

Children at elevated risk for ASD exhibit heterogeneity in symptomatology, other behaviors relevant to ASD, and outcomes. Among high-risk siblings, early behavior often predicts diagnosis, but these patterns of prediction are not clear. We aimed to refine our understanding of heterogeneity in early behavior relevant to ASD by examining the role of common genetic variants. We examined the association between common variants related to dopaminergic functioning and initiating joint attention (IJA). High-risk siblings with *DRD4* and *DRD2* genotypes linked to less efficient dopaminergic functioning exhibited lower levels of IJA than high-risk siblings with variants linked to more efficient dopaminergic functioning. To our knowledge, this is the first investigation of these genetic variants in relation to joint attention in siblings at risk for ASD.

For high-risk siblings, less efficient dopaminergic functioning (indexed by higher dopamine scores) was associated with less optimal behavior in the first year, i.e., with lower levels of IJA. IJA is important for the development of high-risk siblings—referential communication such as IJA is central to later language and social functioning in children at risk for ASD [Gangi et al., 2014; Ibañez et al., 2012; Malesa et al., 2012]. Early referential communication difficulties likely impact social functioning in children at risk for ASD, and these behaviors appear to be influenced by dopaminergic genotypes. Functioning of the dopaminergic system may be linked to IJA through its role in areas potentially important for the production of IJA behaviors, such as attentional control (relevant to the ability to shift attention between an object and social partner), social motivation and reward (relevant to whether an infant might find this social interaction rewarding and be motivated to initiate), and motor control (relevant to the ability to physically produce behaviors). This link may allow for early identification of high-risk siblings at greatest risk for behavioral difficulties in this area.

Although higher dopamine scores were associated with less optimal behavior among high-risk siblings, the opposite pattern emerged in low-risk siblings. Low-risk siblings with higher dopamine scores exhibited *higher* levels of IJA. This pattern suggests differential susceptibility, the hypothesis that children vary in their susceptibility to both adverse and beneficial effects of

their environments [Belsky, 2005; Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007; Belsky & Pluess, 2009]. Common genetic variants have been identified as potential susceptibility factors that modify individuals' susceptibility to influences affecting outcomes. The variants in the current study, *DRD4* and *DRD2*, act as susceptibility genes in multiple contexts [e.g., Bakermans-Kranenburg & van IJzendoorn, 2006, 2011; Sheese, Voelker, Rothbart, & Posner, 2007; Van IJzendoorn & Bakermans-Kranenburg, 2006], but have not been examined in the context of familial risk for ASD.

Although the differential susceptibility hypothesis is often conceptualized as susceptibility to rearing, it may also encompass sensitivity to a broader range of influences [Belsky & Pluess, 2009]. For example, stronger associations between difficult child temperament and externalizing problems are found in children who have older siblings [Mesman et al., 2009]. Endogenous factors have also been conceptualized as internal environments that affect the relationship between genes and outcomes [Schmidt, Fox, Perez-Edgar, & Hamer, 2009]. That is, factors *within* an individual may play a role in moderating the association between genotype and developmental outcomes.

Familial risk for ASD confers increased risk for ASD and related sub-clinical deficits to younger siblings of children diagnosed with ASD. Within a differential susceptibility framework, we conceptualize familial ASD risk as a functional context. Familial ASD risk likely encompasses a combination of unknown factors that presumably are genetic and perhaps environmental, to which children may be more or less susceptible, such as CNVs linked to ASD susceptibility or exposure to environmental toxins [Hallmayer et al., 2011; Newschaffer et al., 2012; Yuen et al., 2015]. Here, high-risk siblings with alleles linked to less efficient dopaminergic functioning exhibited lower levels of IJA (the predicted pattern), while low-risk siblings did not exhibit this same pattern. In siblings with no alleles linked to less efficient dopaminergic functioning, high- and low-risk siblings exhibited similar levels of IJA. While this finding fits within the framework of the differential susceptibility hypothesis, and both dopaminergic genes have been found to act as susceptibility genes in other contexts, additional research will be necessary to determine the specific factors within familial risk for ASD (and lack of familial risk for ASD) that explain the mechanism through which dopaminergic functioning is differentially associated with levels of IJA in these groups. Future research could examine additional genetic factors, environmental factors, and parent qualities in high- and low-risk children.

Sample size limited analysis of high-risk siblings with ASD. Twelve high-risk siblings in the study sample were later diagnosed with ASD, a number insufficient for separate analyses. Findings from the current study should be interpreted with caution until replicated with larger sample sizes. Future research aimed at replicating our findings with larger sample sizes would strengthen our findings of a relationship between dopaminergic genotypes and ASD-relevant behavior and could profitably investigate this relationship among high-risk children with ASD outcomes.

Analyses of *DRD4* and *DRD2* genes individually suggest that *DRD4* genotype may be more strongly associated with IJA than *DRD2* genotype. In addition to the common dopaminergic variants in *DRD4* and *DRD2* examined in the current study, other dopaminergic variants might be examined in future investigations of behavioral characteristics of high-risk siblings and children with autism. For example, a VNTR in the *DAT1* gene is associated with expression of the dopamine transporter [Fuke et al., 2001]. Together, these variants might provide a more comprehensive index of dopaminergic functioning. Additionally, information about copy number variations (CNVs) associated with ASD was not available for the participants in this study, and future research including known pathogenic CNVs may be able to examine their potential role in behavioral heterogeneity in high-risk siblings.

Genotypes outside the dopaminergic system may also impact the outcome of dopaminergic functioning and might further our understanding of dopamine's role in behavioral outcomes. For example, catechol-O-methyl transferase (encoded by the *COMT* gene) is an enzyme that degrades catecholamines including dopamine, and a polymorphism in the *COMT* gene is associated with dopaminergic function [Chen et al., 2004]. Brain-derived neurotrophic factor (BDNF; coded for by the *BDNF* gene) may influence dopamine activity as well [Goggi, Pullar, Carney, & Bradford, 2003; Narita, Aoki, Takagi, Yajima, & Suzuki, 2003; Savitz, Solms, & Ramesar, 2006]. Serotonergic function may also interact with dopaminergic function to influence behavioral outcomes. Levels of dopaminergic functioning might influence sensitivity to reward, leading to either high or low motivation toward rewards. Dopaminergic functioning might then interact with levels of serotonergic functioning influencing effortful control, which could aid in regulation of approach tendencies related to reward sensitivity [Carver, Johnson, & Joormann, 2009].

In the current study, we found that dopaminergic risk genotypes were associated with lower levels of IJA in high-risk siblings. Given the systems in which dopamine plays a role, dopaminergic functioning could potentially affect children's social motivation, reward sensitivity, attention coordination, and even motor control. High-risk siblings with lower dopaminergic functioning appear to exhibit less optimal behavior in early social interaction in the first year.

As the search for replicable genes associated with ASD risk is ongoing, an approach investigating genes that may be relevant to specific behaviors important for the development of children at risk for ASD may be a productive avenue of research. Genes that may not be associated with ASD itself may still be linked to particular behaviors. In addition to aiding identification of high-risk siblings at greatest risk for difficulties, findings may also aid in identifying resilient children. High-risk siblings with fewer genes associated with less efficient dopaminergic functioning were exhibiting fewer difficulties in IJA than high-risk siblings carrying more genotypes associated with less efficient dopaminergic functioning.

Referential communication is associated with ASD symptoms and outcome. Links between dopaminergic variants and behavioral phenotypes relevant to ASD, such as joint attention, can aid in understanding the developmental heterogeneity of high-risk siblings. Identification of common genetic variants—assessable at birth—that confer increased risk for ASD-relevant behaviors has the potential to aid in assessing risk and informing preventive interventions. If replicated, the current results suggest that genotype screening could aid in identifying siblings at the greatest risk for difficulties in areas relevant to later outcomes, even before the emergence of delays or difficulties. Developmental psychopathology could benefit from utilizing genetic markers with documented roles in healthy and problematic behaviors to assess risk and inform preventive interventions.

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