Evaluating heterogeneity in ASD symptomatology, cognitive ability, and adaptive functioning among 16p11.2 CNV carriers

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Individuals with 16p11.2 copy number variant (CNV) show considerable phenotypic heterogeneity. Although autism spectrum disorder (ASD) is reported in approximately 20–23% of individuals with 16p11.2 CNVs, ASD-associated symptoms are observed in those without a clinical ASD diagnosis. Previous work has shown that genetic variation and prenatal and perinatal birth complications influence ASD risk and symptom severity. This study examined the impact of genetic and environmental risk factors on phenotypic heterogeneity among 16p11.2 CNV carriers. Participants included individuals with a 16p11.2 deletion ($N = 96$) or duplication ($N = 77$) with exome sequencing from the Simons VIP study. The presence of prenatal factors, perinatal events, additional genetic events, and gender was studied. Regression analyses examined the contribution of each risk factor on ASD symptomatology, cognitive functioning, and adaptive abilities. For deletion carriers, perinatal and additional genetic events were associated with increased ASD symptomatology and decrements in cognitive and adaptive functioning. For duplication carriers, secondary genetic events were associated with greater cognitive impairments. Being female sex was a protective factor for both deletion and duplication carriers. Our findings suggest that ASD-associated risk factors contribute to the variability in symptom presentation in individuals with 16p11.2 CNVs. ***Autism Res*** *2020, 13: 1300–1310.* © 2020 International Society for Autism Research, Wiley Periodicals, LLC.

Lay Summary: There are a wide range of autism spectrum disorder (ASD) symptoms and abilities observed for individuals with genetic changes of the 16p11.2 region. Here, we found perinatal complications contributed to more severe ASD symptoms (deletion carriers) and additional genetic mutations contributed to decreased cognitive abilities (deletion and duplication carriers). A potential protective factor was also observed for females with 16p11.2 variations.

Keywords: 16p11.2 deletion; 16p11.2 duplication; autism spectrum disorder; individual variability/heterogeneity; cognitive functioning; adaptive functioning

Introduction

Copy number variations (CNVs) in the ~600 kb BP4-BP5 region of 16p11.2 are associated with autism spectrum disorder (ASD), schizophrenia, mood disorders, attention deficit and hyperactivity disorder, intellectual disability, language disorders, motor disorders, obesity, and other neurodevelopmental disorders [Niarchou et al., 2019a; Niarchou et al., 2019b; Kumar et al., 2008; McCarthy et al., 2009; Weiss et al., 2008; Zufferey et al., 2012]. For subjects with a psychiatric or language disorder, frequency estimates are 0.1% and 0.04% for 16p11.2 deletions and duplications, respectively, while accounting for approximately 1% of those clinically diagnosed with ASD. 16p11.2 CNVs are also present and potentially asymptomatic in about 0.01–0.043% of the general population, respectively [Rosenfeld, Coe, Eichler, Cuckle, & Shaffer, 2013; Kirov et al., 2014]. With 16p11.2 CNV carriers, ASD rates are estimated at approximately 25% for deletion carriers and 20% for duplication carriers [Hanson et al., 2015; Snyder et al., 2016]. Within deletion carriers, quantitative assessment across phenotypic domains indicates clinician-observed autism symptom severity ranges from within normal range to severely impaired, with

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comparable variability indicated from parent report [Hanson et al., 2015; Moreno-De-Luca et al., 2015]. A similar pattern of significant ASD-symptom severity variability is observed in duplication carriers [Snyder et al., 2016].

ASD is a behaviorally defined neurodevelopmental disorder characterized by deficits in social communication and interaction and the presence of restrictive and repetitive behaviors (RRBs). Converging evidence provides strong support for the genetic contribution to ASD with hundreds of genomic loci, including 16p11.2, and genes implicated [Coe et al., 2019; Iossifov et al., 2014; Sanders et al., 2015]. Furthermore, the presence of "secondary hits" and multiple genetic risk factors is associated with increased impairment for individuals with ASD [Guo et al., 2018; Girirajan et al., 2012; O'Roak et al., 2011; Samocha et al., 2014]. Studies examining environmental risk factors of ASD have revealed significant associations between prenatal and perinatal risk factors and ASD symptomatology, which may confer independent risk of ASD symptomatology, be the sequelae of genetic insults, or function interactively with other risk factors [Hisle-Gorman et al., 2018; Chien et al., 2019; Gardener, Spiegelman, & Buka, 2011; Langridge et al., 2013; Matelski & Van de Water, 2016; Visser et al., 2013]. Prenatal events such as advanced parental age, preeclampsia, infection or autoimmune disease during pregnancy, and the use of psychotropic or antidepressant medications have been associated with an increased risk of ASD and other neurodevelopmental disorders [Chen et al., 2016; Gardener, Spiegelman, & Buka, 2009; Jiang et al., 2016; Kaplan, Keskin-Arslan, Acar, & Sozmen, 2016; Rais & Rais, 2014; Dachew, Mamun, Maravilla, & Alati, 2018]. Perinatal events such as abnormal (breech) presentation, low birthweight, preterm birth, low 5-min Apgar score, and respiratory distress have also been associated with an increased risk of ASD [Gardener et al., 2011; Modabbernia, Mollon, Boffetta, & Reichenberg, 2016]. Investigations examining interactions between genetic risk and a prenatal risk factor, maternal infection during pregnancy, and increased ASD severity suggest an additive effect of multiple events on the severity of ASD symptomatology [Mazina et al., 2014], across a wide variety of CNV loci.

The study of variability in the phenotypic expression of 16p11.2 implicates the presence of secondary CNVs [Duyzend et al., 2016] and contributions of family background to the observed variability [D'Angelo et al., 2016; Moreno-De-Luca et al., 2015]. In ASD more broadly, pre- and peri-natal factors have been proposed as risk factors in the development of ASD symptoms [Gardener et al., 2009; Gardener et al., 2011; Langridge et al., 2013] and examined as predictors for variability in autism symptomatology [Matelski & Van de Water, 2016; Perrone-McGovern, Simon-Dack, & Niccolai, 2015; Schrieken et al., 2013]. Similarly, within ASD the phenotype varies with sex; females with an ASD diagnosis show less severe symptomatology relative to males with ASD and are less likely to be diagnosed with ASD than males, suggesting a female-protective effect [Krumm et al., 2015; Park et al., 2016; Polyak, Rosenfeld, & Girirajan, 2015]. These reported gender differences in the phenotypic presentation have been associated with secondary genetic hits [Jacquemont et al., 2014] and differences in recognition and diagnostic practices [Constantino & Charman, 2012; Kreiser & White, 2014; Lai, Lombardo, Auyeung, Chakrabarti, & Baron-Cohen, 2015].

To expand on these studies of 16p11.2 and incorporate findings from the literature within the field of ASD, we investigated the impact of other genetic and environmental factors that could predict phenotypic heterogeneity among 16p11.2 deletion and duplication carriers. Specifically, we examined the contribution of secondary genetic events, pre- and peri-natal events, and sex on the variability of ASD symptomatology. We focused on ASD symptomatology given the consistent findings of high rates of ASD diagnosis within this population, as well as consistent social-communication deficits and reports of repetitive interests and behaviors in individuals not meeting diagnostic criteria [Hanson et al., 2015; Snyder et al., 2016].

Methods

Participants

Prior to additional genetic screening, subjects included individuals with the recurrent 600 kb BP4–BP5 16p11.2 duplication or deletion participating in the Simons Variation in Individuals Project [Simons-VIP; Simons VIP Consortium, 2012]. All participants were fluent in English and did not have other genetic diagnoses, additional clinically recognized CNVs, or severe neurological insults. Identified probands and their family members received cascade genetic testing, yielding an initial population size of 137 deletion carriers and 129 duplication carriers (final population characterized is shown in Table 2). See Simons VIP Consortium [2012] for data collection methods. As part of participation in the consortium, informed consent was obtained for all participants in accordance with the ethical standards of each local institutional research review board and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Measures

Additional genetic factors. Sequencing to identify additional rare CNVs was conducted using Illumina HumanOmniExpress microarray platforms [see Duyzend et al., 2016 for methods]. Exome sequencing data from the Simons Variation in Individuals Project were utilized to assess genome-wide exonic variation (as described on

SFARI website; http://sfari.org). We analyzed a total of 431 exomes from the Simons VIP. We applied several filters to the variants present in the exome VCF available through SFARI base using the VCFlib [Garrison, 2012 VCFlib] and VCFtools [Danecek et al., 2011] software suites. For filtering we selected only variants with a PASS flag, depth >10, QUAL >20, removed dbSNP sites excluding sites after build 129, removed Mills and 1,000 genomes gold standard indels, removed segmental duplications and tandem repeats, removed variants found at >1% frequency in dbSnp144, and removed sex chromosomes. We annotated using SeattleSeq build 138, selected the transcript with the most severe annotation for the variant, and retained only non-synonymous and splice variants. We additionally annotated each variant with the Combined Annotation Dependent Depletion (CADD) v1.3 score [Kircher et al., 2014], the allele frequency from the Exac database containing nonpsychiatric cases (ftp://ftp.broadinstitute.org/pub/ExAC_release/release0.3/subsets/ExAC.r0.3.nonpsych.sites.vcf.gz), and the Residual Variation Intolerance Score [Petrovski, Wang, Heinzen, Allen, & Goldstein, 2013; version 3_12_Mar16, columns all 0.1% and %All 0.1%] Tables S1–S3 summarize CNV and SNV results for individuals with sequencing data.

Sequencing results yielded a final study population of 96 deletion carriers and 77 duplication carriers. The presence of additional rare CNVs [defined as occurring with <0.1% frequency in control individuals in the Duyzend et al., 2016 study] and deleterious SNVs (defined as an SNV with a CADD score of >20) were tallied for each individual. Additional SNVs with unknown significance (CADD score = <20) are included in Table S3, but these variants were not included in the frequency of SNVs. Each individual was categorized as having 1 or more additional CNVs, SNVs, both, or none.

Prenatal and perinatal complications. Prenatal risk factors and perinatal events were identified for each participant through parent/caregiver interview. These events were identified as having an increased risk for ASD diagnosis based on findings from meta-analyses conducted by Gardener et al. [2009] and Gardener et al. [2011] for prenatal and perinatal events that demonstrated a positive association with ASD diagnosis or symptomatology. Table 1 lists the prenatal risk factors and perinatal events that were collected in the Simons VIP sample and selected for analysis. The number of events was calculated for each individual yielding a single value ranging from zero to nine.

Phenotypic measurement. ASD symptomatology was based on scores obtained from the Autism Diagnostic Observation Schedule (ADOS), which was administered by trained clinicians to all study participants [Gotham, Pickles, & Lord, 2009]. We used the overall ADOS calibrated severity score (CSS), the social affect (SA) CSS, and the RRB CSS to highlight different domains of the ASD phenotype [Hus, Gotham, & Lord, 2014]. The ADOS CSS scores range from 1 to 10 with 10 indicating greatest severity and scores over 3 suggestive of an ASD diagnosis.

Cognitive ability was also considered in the analysis and was represented by verbal IQ and nonverbal IQ scores collected with the Wechsler Abbreviated Scale of Intelligence [Wechsler, 1999], the Differential Ability Scales [Elliot, 2007], or the Mullen Scales of Early Learning [Mullen, 1995], depending on the age and ability of participants. Similarly, the adaptive ability was collected and measured using the parent interview version of the Vineland Adaptive Behavior Scales (VABS), Second Edition [Sparrow, Balla, & Cicchetti, 2005] with a focus on the overall composite score in the current analysis.

Table 1. Prenatal Risk Factors and Perinatal Events

	16p11.2 deletion			16p11.2 duplication		
	Count	%	*N*	Count	%	*N*
Prenatal risk factors						
1. Advanced maternal age (>35 years)	29	42.0	69	18	27.3	66
2. Advanced paternal age (>40 years)	54	78.3	69	52	78.8	66
3. Preeclampsia	6	7.6	79	5	15.2	33
4. Use of antidepressants	5	7.0	71	2	6.5	31
5. Use of psychotropic medications	1	1.4	71	0	0.0	46
Perinatal events						
1. Low APGAR score at 5 min	2	4.5	44	2	12.5	16
2. Respiratory distress	20	24.1	83	6	17.6	34
3. Supplemental oxygen used	22	27.2	81	5	15.2	33
4. Ventilator used	5	6.3	80	1	2.9	35
5. Abnormal presentation	7	8.6	81	3	5.0	60
6. Preterm labor (<37 weeks of gestation)	18	22.8	79	6	17.6	34
7. C-section performed	29	31.5	92	18	25.0	72
8. Low birthweight (<2,500 g)	11	13.4	82	7	12.1	58

Cognitive and adaptive scores are reported with a mean of 100 and a *SD* of 15.

Given ADOS scores are not normalized as the cognitive and adaptive ability, scores from all measurements were normalized to a *z*-score for comparison across measurements.

Analytic Plan

Chi-square and independent two sample *t*-tests (of unequal variance) were conducted to compare initial demographics, independent variables, and outcome measures between 16p11.2 deletion and duplication carriers. To confirm independence between potential risk variables (sex, perinatal events, prenatal events, SNV w/CADD>20, and Rare CNV), two-tailed correlations were conducted and revealed no significant associations between variables across all participants (r's < 0.24, P's > 0.084) or within each carrier group (deletion carriers, r's < 0.30, P's > 0.10; duplication carriers, r's < 0.34, P's > 0.072) with one exception: Duplication carriers exhibited a positive relationship between the number of deleterious SNVs and number of rare CNV, r = 0.59, P = 0.020.

First, linear mixed-effects models were performed for each carrier group separately to assess the effect of all independent variables on measurements of ASD symptomatology (ADOS CSS, ADOS-SA CSS, and ADOS-RRB CSS) and cognitive and adaptive function (verbal IQ, nonverbal IQ, and VABS). Linear mixed-effects modeling was conducted in order to account for shared variance within the family by including a random intercept for each family. Sex was included in the analyses to ascertain contributions to phenotypic expression. Carrier group differences were directly tested within the second set of linear mixed effect models with identical parameters but an interaction with a group for each factor. All analyses were performed using SAS (version 9.4) using PROC MIXED with maximum likelihood and Satterthwaite denominator degrees of freedom. Standardized coefficients and 95% confidence intervals are provided in Figure 1. Additional post hoc analyses that involved repeating the analyses while controlling for critical factors (NVIQ for ASD symptomology model, ADOS CSS for cognitive and adaptive model) and for ascertainment status (initially identified proband vs. family member) are provided in Supplementary Materials and Figures S1 and S2.

Results

Table 2 describes the study sample; the final study population consisted of 173 individuals from 128 different families. The carrier groups differed in mean age (deletion carriers significantly younger), the frequency of additional genetic events (higher for deletion carriers, though the equal number in probands only; see Table S1), the mean number of perinatal events (higher for deletion carriers), and the mean verbal IQ score (higher for duplication carriers). Deletion and duplication carriers had similar numbers of prenatal risk factors, similar proportions of male participants, and similar nonverbal IQ, Vineland composite, and ASD-symptom severity scores.

ASD Symptomatology

For deletion carriers, an increased number of perinatal events was associated with increased overall ASD symptoms (ADOS CSS: $t(61.4) = 2.08$, $P = 0.042$). Being female was associated with decreased restrictive and repetitive behaviors (ADOS-RRB CSS: $t(80) = -2.79$, $P = 0.007$)). Three trends were noted: Related to secondary genetic hits, first, an increased number of SNVs showed a trend toward increased overall ASD severity (ADOS CSS: $t(81.2) = 1.97$, $P = 0.053$); and second, an increased number of rare CNVs showed a trend in association with increased restrictive and repetitive Behaviors (ADOS-RRB CSS: $t(80) = 1.93$, $P = 0.057$). Third, an increased number of perinatal events showed a trend towards increased social-affect deficits (ADOS-RRB CSS: $t(70.7) = 1.87$, $P = 0.065$).

For duplication carriers, female sex was associated with lower overall ASD symptomatology (ADOS CSS: $t(60) = -2.22$, $P = 0.031$) and decreased social impairment (ADOS-SA CSS: $t(58) = -2.92$, $P = 0.005$). One trend was noted: an increased number of rare CNVs was associated with increased ASD symptomatology (ADOS CSS: $t(60) = 1.82$, $P = 0.074$). No other variables were associated with changes in ASD symptomatology scores for duplication carriers.

Between carrier group differences related to the effects of the variables were noted, such that the effect of sex on social-affect deficits (reduced effect) was stronger for duplication females than deletion, $F(2, 138) = 4.39$, $P = 0.014$. In contrast, the effect of sex on RRBs (reduced effect) was stronger for deletion than duplication females, $F(2, 138) = 3.86$, $P = 0.023$. Lastly, the effect of perinatal events on social-affect deficits (increased effect) was stronger in deletion than duplication carriers, $F(2, 138) = 3.24$, $P = 0.043$.

Cognitive and Adaptive Function

For deletion carriers, increased number of perinatal events was associated with decreases in cognition and adaptive abilities (VIQ: $t(86) = -3.68$, $P < 0.001$; NVIQ: $t(86) = -3.4$, $P = 0.001$; VABS: $t(84) = -2.35$, $P = 0.021$). Additionally, increased number of rare CNVs was also associated with decreases in cognition (VIQ: $t(39.5) = -3.24$, $P = 0.002$; NVIQ: $t(43.9) = -2.92$, $P = 0.006$), but

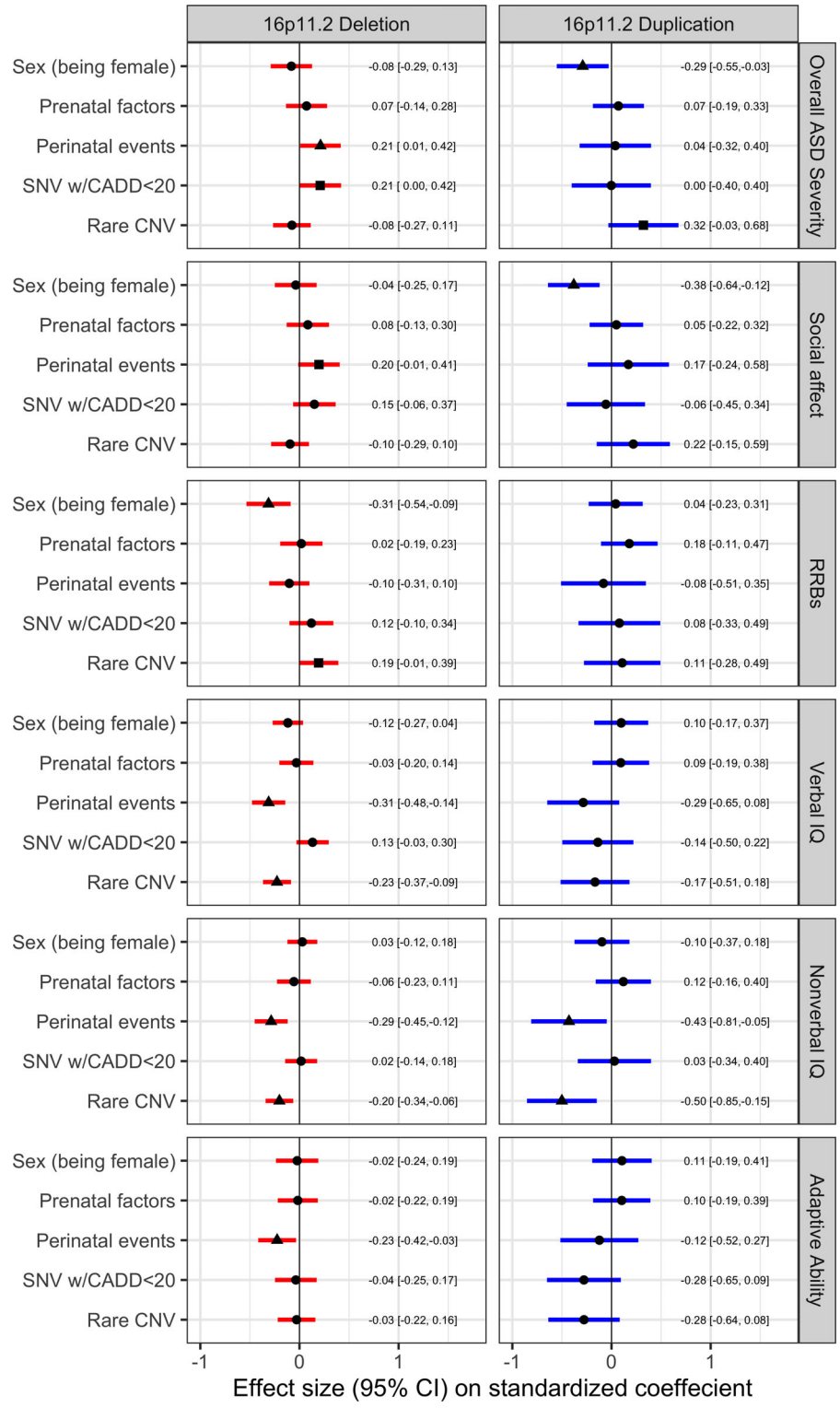

Figure 1. Effects of predictors on ASD symptomology, cognition function, and adaptive function. Results of linear mixed-effects of the five independent variables (sex, perinatal events, prenatal events, SNV w/CADD>20, and rare CNV) for each of the outcome measurements of ASD symptomatology (ADOS CSS score, ADOS-SA CSS score, and ADOS-RRB CSS score), cognitive function (verbal IQ, nonverbal IQ), and adaptive function (Vineland Adaptive Behavior Composite Standard Score). Sex is designated as the effect of being female. The effect of a predictor variable is marked by a central marker, with its 95% confidence interval presented along a horizontal line. Triangular markers indicate a significant effect ($P < 0.05$), squares indicate a trend ($P < 0.1$), and circles indicate a nonsignificant effect.

Table 2. Descriptive Statistics for 16p11.2 Deletion and Duplication Carriers

Characteristics	Deletion	Duplication	*P*-value[a]
Demographic			
N this study	96	77	
Unique families *N*	86 (90%)	42 (54.5%)	
Age in years *M*(*SD*)	10.46 (9.32)	24.99 (19.30)	*<0.001*
Age range in years	0.8–48	1.7–80.1	
Male *N* (%)	73 (53%)	40 (52%)	*0.88*
Clinical ASD diagnosis *N*(%)	22 (22.9%)	10 (14.5%)	*0.25*
Predictor variables (continuous) - Mean number of events (SD)			
Rare CNV	1.1 (1.3)	0.56 (1.0)	*0.003*
SNV w/CADD > 20	0.84 (1.0)	0.49 (0.84)	*0.014*
Prenatal risk factors	1.1 (0.87)	1.1 (0.81)	*0.88*
Perinatal events	1.2 (1.7)	0.67 (1.2)	*0.018*
Genetic factor predictor variables as categorical N = yes(%)			
Both rare CNV and SNV	38 (40%)	17 (22%)	*<0.001*
Rare CNV only	18 (19%)	6 (8%)	
SNV w/CADD > 20	12 (13%)	6 (8%)	
No rare CNV or SNV	28 (29%)	48 (62%)	
Pre- and Peri-natal predictor variables as categorical N = yes(%)			
Both pre- and peri-natal factors	30 (31%)	18 (23%)	*0.20*
Prenatal risk factors only	18 (19%)	9 (12%)	
Perinatal events only	31 (32%)	36 (47%)	
No pre- or peri-natal factors	17 (18%)	14 (18%)	
ASD Symptoms via ADOS CSS[b] *- M(SD)*			
Overall	3.2 (2.3)	3.0 (2.4)	*0.56*
Social affect (SA)	3.5 (2.3)	3.3 (2.5)	*0.55*
Restrictive and repetitive behaviors (RRBs)	3.7 (2.8)	3.5 (2.6)	*0.77*
Cognitive and adaptive functioning - M(SD)[c]			
Verbal IQ	79.4 (18.4)	87.0 (23.9)	*0.023*
Nonverbal IQ	87.5 (16.7)	84.8 (23.1)	*0.38*
Vineland Adaptive Behavior Scales (VABS)	78.7 (13.1)	81.0 (15.6)	*0.31*

[a]One-way analysis of variance (of unequal variance) conducted for comparison of means and χ^2 test conducted for comparison of percentages. All significant *P*-values < .05 are given in italics.

[b]ADOS Calibrated Severity Score, standardized 1–10 scoring with 1–3 indicating non-ASD and 4–10 indicating ASD.

[c]Verbal IQ, nonverbal IQ, and Vineland scores are standardized to a mean = 100, *SD* = 15.

not adaptive abilities (VABS: $t(84) = -0.31$, $P = 0.79 = 6$). No other variables were associated with changes in cognitive and adaptive function for deletion carriers.

For duplication carriers, none of the independent variables was associated with changes in standardized verbal IQ scores or adaptive skills. Similar to deletion carriers, decreased nonverbal cognition was associated with increased number of rare CNVs (NVIQ: $t(62.9) = -2.25$, $P = 0.028$) and an increased number of perinatal events (NVIQ: $t(62.5) = -2.84$, $P = 0.006$).

Between carrier group differences related to the effects of the variables were noted (see Fig. S2), such that the estimated effects of increased perinatal events on verbal and nonverbal cognition (reduced effect) was stronger for duplication than deletion carriers [VIQ: $F(2,130) = 5.07$, $P = 0.0076$; NIVQ: $F(2,136) = 6.01$, $P = 0.0032$]. The estimated effect of increased rare CNVs was stronger for deletion carriers in verbal cognition (VIQ: $F(2,142) = 4.81$, $P = 0.0095$) but stronger for duplication carriers in nonverbal cognition (NVIQ: $F(2,145) = 7.24$, $P = 0.001$).

Discussion

This study investigated the impact of secondary genetic events, prenatal and perinatal environmental factors, and sex on phenotypic heterogeneity among 16p11.2 CNV carriers. Critically, despite a lack of group-level differences in ASD symptomatology, nonverbal cognition, and adaptive functioning between carrier groups, we found differential contributions of our risk factors for each carrier group. Broadly, we identified patterns suggesting that overall ASD symptomology was influenced by the frequency of perinatal events in deletion carriers. ASD symptomology was influenced by sex in duplication carriers, such that those of female sex showed significantly less ASD-related deficits, specifically within social-affect symptoms, whereas female deletion carriers exhibited restricted and repetitive behaviors. The frequency of perinatal events and the presence of additional CNVs conferred additional deficits in nonverbal cognition in both deletion and duplication carriers, with increased perinatal

events being associated with reduced verbal cognition and adaptive abilities in deletion carriers.

Perinatal contributions. Our results support previous reports of the interactive effects of perinatal events and CNVs on increased ASD symptomatology and reduced cognitive and adaptive behaviors [Mazina et al., 2014], specifically for 16p11.2 deletion carriers. Importantly, the effect of perinatal events remained in models controlling for nonverbal cognition (see Fig. S1). Deletion carriers had significantly more perinatal events (40% deletion vs. 22% duplication), which lends greater variability and power to detect relationships between risk factors and outcomes. The lack of a perinatal contribution to ASD symptomology in duplication carriers may be related to the reduced severity phenotype commonly observed [Steinman et al., 2016; Niarchou et al., 2019a; Niarchou et al., 2019b], yet, we caution over-interpretation of the lack of an association, given the limited sample size of this exploratory study. In addition, although an item-level analysis was beyond the scope of this study, continued work should specifically investigate high-frequency perinatal events: respiratory distress, supplemental oxygen used, preterm labor, and Caesarean section performed. These elements have been previously noted as risk factors for both ASD and intellectual disability [see Modabbernia et al., 2016 for a review], and it is likely that these events stem from a common underlying cause. However, the relative contribution of each event, as well as patterns related to certain constellations of perinatal events, should be explored in future studies.

Secondary genetic contributions. While rare CNVs were contributing factors of cognition for both deletion carriers (verbal, nonverbal) and duplication carriers (nonverbal only), secondary genetic factors were weaker in regard to ASD symptomology. Two trends within the deletion-carrier group indicated that deleterious SNV and rare CNVs may impact overall ASD severity and RRBs, respectively. In addition, when cognitive models controlled for ASD severity (via ADOS CSS, see Fig. S2), an increased number of additional rare CNVs accounted for reduced verbal and nonverbal cognition. These findings are aligned with prior work emphasizing a strong role of more severe mutations (e.g., de novo mutations, rare CNVs, and deleterious SNVs) on intellectual and cognitive capacity [Girirajan et al., 2010; Hamdan et al., 2014].

There is clearly a complex phenotype–16p11.2 genotype relationship with recent work suggesting that 16p11.2 deletions may lead to intellectual impairments directly, yet ASD indirectly (i.e., ASD due to reduced cognitive abilities) [Moreno-De-Luca, Moreno-De-Luca, Cubells, & Sanders, 2014]. However, although females with ASD show a higher mutational burden and lower cognitive abilities, adjustments for cognition do not change the excess mutational burden [Jacquemont et al., 2014], indicating that these effects are not solely related to cognition. Moving forward toward clinical trials, it will be important to emphasize cross-level interactions to understand the mechanism by which cognitive abilities may be impacted by secondary genetic factors. For instance, evidence from neuroimaging suggests divergent mechanisms by which 16p11.2 carriers process information [Hudac et al., 2015], which may further describe how cognitive abilities may support (or interfere with) the social phenotype, broadly, and more specifically, ASD symptomology.

As a caveat, prior work indicated a similar number of secondary de novo CNVs, often regarded as more pathogenic, in 16p11.2 probands specifically [Duyzend et al., 2016]. Although our sample seemingly illustrates larger numbers of potentially disruptive genetic events than duplication carriers (71% deletion vs. 38% duplication, see Table 2), these effects may be inflated due to the lack of additional events in family members. When we specifically examine rates of potentially disruptive genetic events in the initially identified probands (Table S1), there are a similar number of rates between deletion and duplication probands (81% deletion and 88% duplication). However, results in probands only (Fig. S2) support the hypothesis that additional genetic factors are contributing to cognitive and adaptive functioning, in particular.

Female protective effect. We also found associations between female sex and less severe ASD symptomatology among both 16p11.2 deletion carriers (RRB subdomain) and duplication carriers (overall and SA subdomain). This finding adds additional support for a female protective effect [Robinson, Lichtenstein, Anckarsäter, Happé, & Ronald, 2013] that describes the phenomenon in which females with ASD exhibit less severe ASD symptomology and is less likely to be diagnosed than males. Importantly, sex did not contribute to cognitive or adaptive abilities, suggesting that the female protective effect is specific to ASD symptomology. Previous work using clinical samples has shown that females with diagnoses of ASD have lower IQs and are more severely affected than their male counterparts [Fombonne, 2003]. Within our sample, IQ did not vary based upon sex within carrier group (post hoc, P's > 0.10) or as related to ASD diagnosis (post hoc, P's > 0.17), though a trend indicated reduced nonverbal cognition in female duplication carriers with ASD (post hoc, P's = 0.061). This is related to the analysis indicating the female protective effect for nonverbal cognition in duplications when overall ASD symptomology was controlled (Fig. S1).

Our primary data indicate a reduction of ASD symptoms for females in both carrier groups, yet, there may be additional implications for being female and having

reduced nonverbal cognition. For one, although our study did not consider the interaction between environmental and genetic factors, it is important to begin to understand how the constellation of cross-factors relates to the 16p11.2 phenotype. Robinson et al. [2014] found that females in their clinically based sample had nearly twice the number of loss of function mutations than males, suggesting that females may require a greater etiological burden to manifest greater ASD symptomatology [Robinson et al., 2013].

Future directions. The five risk factors accounted for 9 to 20% of the variability (R^2 values) in the phenotypic outcome measurements. These findings suggest that while the additional factors that we identified are contributing to the variability in ASD symptoms there are other factors that may account for this variance.

Previous work indicates the strong role of parental background in accounting for this variance, including contributions from noncarrier parents [Moreno-De-Luca et al., 2015], suggesting a potential dimensional shift related to underlying genetic background. A random intercept was included in this analysis as a way to control for multiple family members, consistent with a previous study that did not find evidence of correspondence of cognitive abilities or social skills between carrier children and carrier parents, [Snyder et al., 2016]. However, we did not explicitly examine relationships between first-degree family members (e.g., initially identified child proband compared to carrier parent, noncarrier parent, and/or biparental mean). It is likely that additional familial factors at both the genetic or environmental levels are contributing to the phenotype. For instance, noncarrier family members exhibit anatomical brain alterations compared to noncarrier nonfamily controls [Martin-Brevet et al., 2018], suggesting a more complex investigation of the possible parental background and additional risk factors is critical to better account for variability.

There are several limitations to note. First, in this study, we examined whether the quantity of prenatal and perinatal events was related to functional outcomes without consideration of specific events. It may be the case that the severity of certain events outweighs other events, which may be evidenced by contrasting findings in the extant literature. For one, an earlier meta-analysis found no association between preeclampsia and ASD risk [Gardener et al., 2009], in contrast with a more recent meta-analysis [Dachew et al., 2018] that indicated preeclampsia as a strong ASD-risk factor. Second, we are limited in our ability to fully assess codependent or shared aspects, such that the factors targeted by these analyses may reflect underlying biology and/or environmental features. For instance, it is difficult to determine prenatal risk factors and perinatal events are truly independent, or rather, may reflect a common unified consequence of interrelated factors (e.g., women with advanced maternal age are more likely to give birth via C-section). Relatedly, several key demographic features were not addressed within our analyses. Of particular note, due to cascade genetic testing commonly used to confirm and subsequently identify additional familial 16p11.2 carriers, a larger proportion of the duplication carriers were related, older adults (see Table 2). These features may have led to increased variance between carriers (i.e., larger confidence intervals), though post hoc analyses with only initially identified probands revealed similar findings (see Fig. S2). Third, our study is exploratory in nature, such that continued work is required to confirm that these differences are not mere detection differences, but instead reflective of underlying mechanisms stemming from 16p11.2 CNVs. Here, effect sizes are provided as confidence intervals around standardized coefficients in order to better represent relative contributions across different factors. However, these values are not meant to be clinically significant but rather descriptive, as more extensive work with larger samples would be required to determine statistical reliability and thus, clinical relevance.

Overall, this study demonstrated support for the contribution of ASD-associated risk factors (perinatal events, sex, and secondary rare CNVs) to increased ASD symptoms and cognitive/adaptive impairment in individuals with 16p11.2 CNVs. These findings are consistent with prior work suggesting that it is the confluence of multiple genetic risk factors that increases the likelihood of neurodevelopmental disorders, more broadly [Girirajan et al., 2012]. Interactions with environmental factors related to social and psychological familial aspects should also be considered [Baker, Devine, Ng-Cordell, Raymond, & Hughes, 2020] as a means to evaluate and understand the gene by environment mechanisms [Wender & Veenstra-Vanderweele, 2017]. As a note of caution, this exploratory study serves as a first foray into specifying specific secondary genetic and environmental factors related to the 16p11.2 CNV phenotype. Future work would benefit from a more careful exploration of the presence of specific environmental and genetic factors.

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Conflict of interest

E.E.E. is an investigator of the Howard Hughes Medical Institute. E.E.E. is on the scientific advisory board (SAB) of DNAnexus, Inc. There are no other conflicts of interest to report.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1: Supporting Information
Table S1 Sequenced Individuals
Table S2: Rare CNV calls (frequency < 0.1% controls) in the 16p11.2 probands screened
Table S3: Private SNV calls