ARCHIVAL REPORT

The Cognitive and Behavioral Phenotype of the 16p11.2 Deletion in a Clinically Ascertained Population

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Background: Deletion of the recurrent ~600 kb BP4-BP5 chromosomal region 16p11.2 has been associated with a wide range of neurodevelopmental outcomes.

Methods: To clarify the phenotype of 16p11.2 deletion, we examined the psychiatric and developmental presentation of predominantly clinically referred individuals, with a particular emphasis on broader autism phenotype characteristics in individuals with recurrent ~600 kb chromosome 16p11.2 deletions. Using an extensive standardized assessment battery across three clinical sites, 85 individuals with the 16p11.2 deletion and 153 familial control subjects were evaluated for symptom presentation and clinical diagnosis.

Results: Individuals with the 16p11.2 deletion presented with a high frequency of psychiatric and developmental disorders (~90%). The most commonly diagnosed conditions were developmental coordination disorder, phonologic processing disorder, expressive and receptive language disorders (71% of individuals >3 years old with a speech and language–related disorder), and autism spectrum disorder. Individuals with the 16p11.2 deletion not meeting diagnostic criteria for autism spectrum disorder had a significantly higher prevalence of autism-related characteristics compared with the familial noncarrier control group. Individuals with the 16p11.2 deletion had a range of intellectual ability, but IQ scores were 26 points lower than noncarrier family members on average.

Conclusions: Clinically referred individuals with the 16p11.2 deletion have high rates of psychiatric and developmental disorders and provide a genetically well-defined group to study the emergence of developmental difficulties, particularly associated with the broader autism phenotype.

Key Words: 16p11.2 deletion, autism, autism spectrum disorder, developmental disability, genetics, psychiatric diagnosis

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gathered from questionnaires completed by referring clinicians, 45 probands from the Simons Variation in Individuals Project (Simons VIP), and 117 through literature review (which included participants ascertained for developmental/intellectual disabilities, obesity, and from the general population). Results revealed that full-scale IQ (FSIQ) scores were 2 SDs lower in carriers relative to familial control subjects, with verbal IQ (VIQ) being generally lower than nonverbal IQ (NVIQ). Also, 15% of carriers were classified as having ASD, many required speech therapy, and >70% were found to have comorbid psychiatric diagnoses. This heterogeneity in phenotypic presentation is also found in other chromosomal CNVs (e.g., 1q21.1) and gene mutations (e.g., NLGN4, NRXN1, SHANK2) associated with ASD and psychiatric disorders (10–15). However, limited phenotypic assessment has been completed in many of these rare genetic disorders, limiting the assessment of the true phenotypic heterogeneity of these disorders.

In these prior studies characterizing 16p11.2 deletion carriers, diagnostic characterization was established through multiple methods, including clinical assessment, questionnaires, and medical history reviews in some cases; this process often lacked a standardization of the clinical assessment. Finally, several of these studies included multiple modes of ascertainment. These limitations stress the need for large sample sizes ascertained uniformly for the presence of the 16p11.2 deletion and assessed with a standardized neuropsychological battery to assess the diversity of difficulties previously found to be common in the disorder as well as standardized assessments of nondeletion relatives to serve as familial control subjects.

Familial comparisons offer a valid design (16–18) as well as an efficient way to overcome potential confounding problems inherent to unrelated case-control designs, including differing genetic backgrounds and socioeconomic statuses (19,20). To characterize in detail psychiatric and developmental problems such as ASD in a genetically well-defined CNV, we performed cognitive, adaptive, language, psychiatric, behavioral, and diagnostic testing, including standardized ASD assessment, on a large number of individuals with 16p11.2 deletions and noncarrier siblings and parents. Because of the high likelihood of ASD in this population, we also explored whether differences between the carriers and familial control subjects were associated with the deficits inherent to this diagnosis or if these deficits were seen even when controlling for ASD-related difficulties.

Methods and Materials

Subjects

Subjects included individuals with the same recurrent 600 kb BP4-BP5 16p11.2 deletion without other pathogenic CNVs or known genetic diagnoses, the biological siblings of the individual with the deletion, and the biological parents of the individual with the deletion (Table 1). Siblings were selected for participation based on closeness in age to the carrier. One half-sibling was included. Adoptive parents were not used as control subjects but were interviewed for information about their carrier child. Most individuals with the 16p11.2 deletion were clinically identified, but cascade genetic testing within the families (see later) identified some additional carriers.

Biological or adoptive families that included an individual with the recurrent ~600 kb 16p11.2 BP4-BP5 deletion mediated by segmental duplications (chromosome 16 position 29,652,999–30,199,351 in hg19) identified through clinical diagnostic evaluations and who expressed interest in participating in research on the Simons VIP Connect website were invited to participate. All deletion carriers had the same recurrent deletion and no additional pathogenic CNVs or known monogenic disorders. Recruitment included directing traffic to the Simons VIP Connect website (SimonsVIPConnect.org) from Google Ads, links from patient advocacy websites and social media sites, collaborations with clinical molecular cytogenetics laboratories that informed treating physicians of the study, and direct mailings to medical professionals. [See Simons VIP Consortium (21) for more details on recruitment and inclusion and exclusion criteria.]

Cascade genetic testing was conducted for all family members using a custom-designed oligonucleotide array containing genome-wide coverage at a resolution of ~400 kb and targeting known disease gene coverage at a resolution of ~50 kb (OGT 60K; Oxford Gene Technologies, Tarrytown, New York), according to previously published methods of analysis (22), to determine if the deletion was de novo or inherited and to identify other deletion carriers within the family.

Following screening, families participated in data collection at one of three Simons VIP phenotyping sites (Boston, Houston, and Seattle) for a comprehensive and standardized multiday evaluation. The study was approved by the institutional review board at each participating institution; all participants provided informed consent before data collection. All diagnostic interviewing and cognitive testing of children <5 years old was videotaped for later review. Standardization of measurement across sites included mandatory formalized, standardized training on all measures through in-person training sessions and webinars for all clinicians, cross-site reliability and maintenance through monthly clinician conference calls and periodic videotape review, and validation and diagnostic confirmation through data review and observation of video recorded sessions by independent consultants.

The current analyses were limited to individuals ≥3 years old because of lack of complete data sets in very young children and infants; to control for the possible instability of IQ measurement in very young children, particularly children with ASD and developmental disability (23–28); and to control for changes in presentation and rates of DSM diagnoses over time in very young children (29–31).

Phenotypic Assessment

Psychiatric Diagnosis. Experienced, licensed clinicians gave best-estimate, clinical DSM-IV-TR (32) diagnoses using all information obtained during the research evaluation. Information was based on the standardized interview, questionnaire, and observation processes described subsequently as well as results from standardized administration of the Diagnostic Interview Schedule for Children (33) and symptom checklist 90 (34) and review of available medical records and prior testing. To capture the range of psychiatric presentation, exclusionary criteria for diagnoses were not considered (e.g., if a child met criteria for both attention-deficit/hyperactivity disorder [ADHD] and ASD, both diagnoses were considered). Autism-specific diagnostic measures included the Autism Diagnostic Observation Scale (35) and the Autism Diagnostic Interview—Revised (36). Severity of ASD symptoms was calculated using the Calibrated Severity Score (37).

Autism-Related Symptom Measures. The Social Responsiveness Scale (SRS) was completed by parents about their children with the 16p11.2 deletion and the designated siblings (38). Total raw scores from the SRS were calculated and used within analyses for children 4–18 years old. Raw scores were used to provide greater differentiation of scores at the lower and higher end of...
<table>
<thead>
<tr>
<th>Measure Employed</th>
<th>16p11.2 Carrier</th>
<th>Noncarrier</th>
<th>Carrier vs. Noncarrier</th>
<th>Carrier vs. Noncarrier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X Age ± SD</td>
<td>X Age ± SD</td>
<td>p Value Not Controlling</td>
<td>p Value Not Controlling</td>
</tr>
<tr>
<td>VABS-II</td>
<td>10.2 ± 7.3</td>
<td>11.2 ± 4.9</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Composite</td>
<td>80.0 ± 18.2</td>
<td>104.2 ± 10.8</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Social</td>
<td>81.4 ± 14.9</td>
<td>103.6 ± 10.0</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Communication</td>
<td>77.4 ± 13.4</td>
<td>104.2 ± 10.7</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Daily Living</td>
<td>82.2 ± 13.4</td>
<td>104.4 ± 11.8</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>IQ</td>
<td>11.1 ± 8.9</td>
<td>30.5 ± 14.2</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Verbal IQa</td>
<td>79.0 ± 18.0</td>
<td>106.6 ± 11.9</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Nonverbal IQa</td>
<td>86.8 ± 15.1</td>
<td>110.3 ± 13.2</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
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<tr>
<td>Full-Scale IQa</td>
<td>82.7 ± 15.0</td>
<td>109.5 ± 12.3</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>CASL (Ages 3–12 Years)</td>
<td>9.6 ± 3.8</td>
<td>10.6 ± 4.2</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>EHI</td>
<td>8.8 ± 4.0</td>
<td>12.5 ± 8.4</td>
<td>.0002</td>
<td>.03</td>
</tr>
<tr>
<td>CBCL (Ages 6–18 Years)</td>
<td>10.5 ± 2.9</td>
<td>11.4 ± 3.5</td>
<td>&lt;.0001</td>
<td>.01</td>
</tr>
<tr>
<td>Total T</td>
<td>61.2 ± 10.8</td>
<td>47.0 ± 10.7</td>
<td>&lt;.0001</td>
<td>.01</td>
</tr>
<tr>
<td>Internalizing T</td>
<td>60.4 ± 10.0</td>
<td>48.8 ± 11.0</td>
<td>&lt;.0001</td>
<td>.03</td>
</tr>
<tr>
<td>Externalizing T</td>
<td>54.3 ± 11.1</td>
<td>47.8 ± 8.8</td>
<td>.006</td>
<td>.54</td>
</tr>
<tr>
<td>Conduct Problems T</td>
<td>56.1 ± 7.0</td>
<td>52.2 ± 4.9</td>
<td>.002</td>
<td>.37</td>
</tr>
<tr>
<td>ADHD Problems T</td>
<td>62.3 ± 8.1</td>
<td>53.4 ± 4.8</td>
<td>&lt;.0001</td>
<td>.0002</td>
</tr>
<tr>
<td>WIAT (First Grade and Beyond)</td>
<td>13.9 ± 9.5</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Word Reading</td>
<td>75.5 ± 15.6</td>
<td></td>
<td></td>
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<tr>
<td>Reading Comprehension</td>
<td>83.5 ± 16.4</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sentence Composition</td>
<td>79.4 ± 16.5</td>
<td></td>
<td></td>
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<tr>
<td>Numerical Operations</td>
<td>79.9 ± 17.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTOPP Non-Word Repetition (Ages 5–24 Years)</td>
<td>10.1 ± 3.5</td>
<td>12.7 ± 7.6</td>
<td>&lt;.0001</td>
<td>.01</td>
</tr>
<tr>
<td>BSIQ</td>
<td>5.4 ± 2.3</td>
<td>7.8 ± 2.4</td>
<td>&lt;.0001</td>
<td>.02</td>
</tr>
<tr>
<td>SRS Parent Report (Ages 4–18 Years)</td>
<td>8.9 ± 4.0</td>
<td>10.5 ± 4.2</td>
<td>&lt;.0001</td>
<td>.002</td>
</tr>
<tr>
<td>SRS Parent Report (Ages 4–18 Years)</td>
<td>9.3 ± 7.1</td>
<td>2.5 ± 3.4</td>
<td>&lt;.0001</td>
<td>.002</td>
</tr>
<tr>
<td>SRS Parent Report (Ages 4–18 Years)</td>
<td>95 ± 3.4</td>
<td>105 ± 3.9</td>
<td>&lt;.0001</td>
<td>.003</td>
</tr>
<tr>
<td>SRS Parent Report (Ages 4–18 Years)</td>
<td>77.8 ± 32.5</td>
<td>20.9 ± 17.0</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; BSIQ, Behavior and Sensory Interests Questionnaire; CASL, Comprehensive Assessment of Spoken Language; CBCL, Child Behavior Checklist; CTOPP, Comprehensive Test of Phonological Processing; EHI, Edinburgh Handedness Inventory; NVIQ, nonverbal IQ; SRS, Social Responsiveness Scale; T, T score; VABS-II, Vineland Adaptive Behavior Scales, Second Edition; WIAT, Wechsler Individual Achievement Test.

aNot controlled for NVIQ.
the scales. The SRS-Adult version was completed for each parent by a spouse or partner (39). The Behavior and Sensory Interests Questionnaire (BSIQ) was also completed by parents about their children with the deletion and designated siblings. The BSIQ is an 87-item interview assessing repetitive and stereotyped interests and behaviors.

Cognitive and Behavioral Measures. Participants were administered a developmentally appropriate cognitive measure: Mullen Scales of Early Learning (40); Differential Abilities Scale, Second Edition (41); or Wechsler Abbreviated Scales of Intelligence (42). Standard scores or ratio IQ scores were used to calculate FSIQ, VIQ, and NVIQ, when possible. Other measures included Vineland Adaptive Behavior Scales, Second Edition (parent interview version) (43); Wechsler Individual Achievement Test (44); Comprehensive Assessment of Spoken Language (45); Comprehensive Test of Phonological Processing, Non-Word Repetition (46), Child Behavior Checklist for ages 6–18 (47–49); and Edinburgh Handedness Inventory (50). Only data from individuals who met age and basal criteria stated in the test manuals were used for analysis.

Data Analysis
We examined differences in psychiatric diagnosis, autism-related symptoms, cognitive and adaptive skills, social and language abilities, and behavioral symptoms between 16p11.2 deletion carriers and noncarrier familial control subjects. We used random intercept linear mixed models (LMMs) for continuous outcomes and generalized estimating equations (GEEs) with a compound symmetric correlation structure for categorical and count outcomes. Deletion carriers were divided into three groups: de novo carriers, inherited carriers, and carriers with unknown inheritance status. In all LMMs and GEEs, we estimated the differences in the outcome measures between de novo cases and inherited cases and between each carrier group and noncarrier familial control subjects, while accounting for correlated measures within family. We also constructed a contrast to estimate the differences between all carriers and noncarrier familial control subjects. Group differences were compared, unless otherwise noted, after controlling for age, sex, and NVIQ. To examine the effect of ASD on the behavioral presentation of individuals with 16p11.2 deletion, additional LMM and GEE analyses were conducted controlling for ASD diagnosis. Because of the limited differences found between de novo and inherited carriers or between any inheritance group and carriers with unknown inheritance status as well as the small number of inherited cases, we report the combined differences between all carriers and noncarrier familial control subjects in the main text and show the comparisons between de novo and inherited carriers in Tables S1–S5 in Supplement 1. To account for multiple comparisons, Bonferroni correction was used, yielding a corrected $\alpha$ value of .0018 for the 28 comparisons. All statistical analyses were conducted using SAS Version 9.3 (SAS Institute Inc, Cary, North Carolina), IBM SPSS (Version 19.0, released 2010; IBM Corp., SPSS Statistics for Windows, Armonk, New York), and R 3.0.2 (The R Project for Statistical Computing; http://www.R-project.org/).

Results

Psychiatric Diagnoses
Individuals with the 16p11.2 deletion presented with multiple psychiatric comorbid disorders (Figures S1 and S2 in Supplement 1): 93% of carriers had at least one diagnosis compared with only 21% of control subjects. Developmental coordination disorder, phonological processing disorder, language disorders, and ASD were the most common psychiatric diagnoses observed in carrier participants. Overall, there was a profile of speech and language–based disorders among the 16p11.2 deletion carriers, with 71% of the individuals having a speech and language–related disorder (Table 2). The GEE analysis indicated that individuals with the 16p11.2 deletion had a higher expected number of psychiatric diagnoses even when controlling for NVIQ, age, and sex ($p < .0001$). The mean number of distinct diagnoses in noncarriers was .3, whereas individuals with the deletion had an average of 2.9 diagnoses (a nearly 10-fold increase in number) (Table 2). These differences in expected number of diagnoses persisted after controlling for ASD diagnosis ($p < .0001$) (Table S3 in Supplement 1).

Cognitive Ability
As shown in Table 1, individuals with the 16p11.2 deletion demonstrated an average FSIQ score of 82.7, representing a 26.8-point (1.8 SD) shift downward compared with the FSIQ average of 109.5 in noncarrier control subjects. The same pattern was observed for NVIQ and VIQ. In line with their increased odds of a diagnosis related to above-mentioned language difficulties, 27% of participants with the deletion demonstrated a VIQ < NVIQ discrepancy of $\geq$15 points, whereas only 7% showed a pattern of NVIQ < VIQ. This pattern is consistent across ages and includes adults (Figure 1).

Autism-Related Symptoms
Social Functioning. Children 4–18 years old with the 16p11.2 deletion had significantly higher SRS total scores compared with control subjects (Table 1), even when controlling for the presence of ASD.

Restricted and Repetitive Behaviors. Almost all children with 16p11.2 deletion had some reported level of restricted and repetitive behavior patterns, with 88% of carriers versus 33% of control subjects reporting more than two of these types of behaviors. There was a significant difference in total BSIQ scores for restricted and repetitive behaviors in individuals with the 16p11.2 deletion compared with noncarriers, which decreased slightly when controlling for age, sex, NVIQ, and ASD (Table 1).

Adaptive Skills. Individuals with the 16p11.2 deletion exhibited a wide range of adaptive skill abilities on both composite and subdomain (Social, Communication, Daily Living Skills) scores. Poorer abilities in individuals with 16p11.2 deletion relative to noncarriers were found across all subdomains when controlling for age, sex, and NVIQ (Table 1).

Academic Skills
On the Wechsler Individual Achievement Test, school-age children and adults with the 16p11.2 deletion performed in the below-average to borderline range across reading comprehension, word reading, sentence composition, and numerical operations, on average (Table 1). In basic word reading, 79% fell at least 1 SD (SD = 15) below the test mean of 100, whereas 67% fell $\geq$2 SDs below the test mean. Of carriers, 65% scored at least 1 SD below the test mean in math (numerical operations subtest), and 32% of those individuals were lower by $\geq$2 SDs. A discrepancy of at least 1 SD in between reading or math achievement scores and FSIQ was shown in 31% of individuals with the deletion.
Language Ability

Overall Comprehensive Assessment of Spoken Language scores for individuals with the 16p11.2 deletion were significantly lower than scores for noncarrier control subjects. On the Non-Word Repetition task from the Comprehensive Test of Phonological Processing, there was a significant difference between verbal individuals with 16p11.2 deletion and familial control subjects, which decreased slightly when controlling for age, NVIQ, sex, and ASD diagnosis (Table 1).

Behavioral Difficulties

Analyses revealed that mean T-scores on the Child Behavior Checklist for ages 6–18 for children with 16p11.2 deletion were higher (more impaired) than T-scores for control siblings on the total Child Behavior Checklist score, internalizing domain score, ADHD, and affective problems subscales. Of the children who were deletion carriers, 46% showed problems in the clinically significant range on the total-problem scale; 23%, on externalizing problems; and 38%, on internalizing problems. A significant difference in odds of ADHD diagnosis between carriers and control subjects persisted even after controlling for NVIQ, age, sex, and ASD (Table 1).

Handedness

Handedness information based on the Edinburgh Handedness Inventory was available for 75 deletion carriers (including all ages) and 38 noncarrier family members. Of carriers, 19% reported left hand dominance and 29% reported mixed dominance compared to 3% and 11% of noncarrier family members, respectively. Significantly higher odds of left hand or mixed dominance for deletion carriers were revealed by GEE (Table 1).

Site Effects

Across all quantitative dimensional measures used, after controlling for multiple comparisons, there were no significant differences between sites. Across diagnostic categories, there were differences across the sites with regard to the rate at which developmental coordination disorder ($p < .0002$), language disorder ($p = .003$), and enuresis disorder ($p = .02$) were diagnosed. However, the same pattern of commonly occurring diagnoses was observed. As noted in the Methods and Materials section, analyses of the differences between the de novo and

**Table 2. Frequency of DSM-IV Psychiatric Disorders**

<table>
<thead>
<tr>
<th>Disorder Type</th>
<th>Child Carrier (Age 3–17)</th>
<th>Adult Carrier</th>
<th>Noncarrier Child (Age 3–17)</th>
<th>Noncarrier Adult</th>
<th>Carrier vs. Noncarrier Age, Sex, NVIQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phonological Processing Disorder (315.39)</td>
<td>44 (56%)</td>
<td>0</td>
<td>3 (8%)</td>
<td>0</td>
<td>.0001</td>
</tr>
<tr>
<td>Developmental Coordination Disorder (315.4)</td>
<td>45 (58%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>.0002</td>
</tr>
<tr>
<td>Language Disorders (315.31, 315.32)</td>
<td>36 (46%)</td>
<td>1 (14%)</td>
<td>1 (3%)</td>
<td>0</td>
<td>.0001</td>
</tr>
<tr>
<td>Enuresis (307.6)</td>
<td>16 (21%)</td>
<td>0</td>
<td>2 (5%)</td>
<td>0</td>
<td>.0002</td>
</tr>
<tr>
<td>Autism Spectrum Disorders (299.00, 299.80)</td>
<td>20 (26%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>.03</td>
</tr>
<tr>
<td>ADHD Diagnosis (314.00, 314.01, 314.9)</td>
<td>15 (19%)</td>
<td>0</td>
<td>3 (8%)</td>
<td>3 (3%)</td>
<td>.01</td>
</tr>
<tr>
<td>Borderline Intellectual Functioning (v62.89)</td>
<td>10 (13%)</td>
<td>2 (29%)</td>
<td>0</td>
<td>3 (3%)</td>
<td>.22</td>
</tr>
<tr>
<td>Intellectual Disability (317, 318, 319)</td>
<td>8 (10%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Behavior Disorders (312.9, 313.82)</td>
<td>10 (13%)</td>
<td>0</td>
<td>1 (3%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Learning Disorders (315.0, 315.1, 315.2, 315.9)</td>
<td>10 (13%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Anxiety Disorders (300.0, 300.02, 300.4, 300.9)</td>
<td>5 (6%)</td>
<td>0</td>
<td>3 (8%)</td>
<td>10 (9%)</td>
<td></td>
</tr>
<tr>
<td>Tic Disorder (307.2, 307.22, 307.3)</td>
<td>5 (6%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Number of Diagnoses</td>
<td>$\bar{x} \pm SD$</td>
<td>$\bar{x} \pm SD$</td>
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<td>$\bar{x} \pm SD$</td>
<td>$\bar{x} \pm SD$</td>
</tr>
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ADHD, attention-deficit/hyperactivity disorder; NA, not available; NVIQ, nonverbal IQ.

![Distribution of FSIQ by Deletion Status](image)

Figure 1. Distribution of full-scale IQ (FSIQ) scores by deletion status highlights a 1.8 SD decrement in scores in 16p11.2 deletion cases relative to noncarrier family members. Dotted vertical line represents cutoff value for intellectual disability.
inherited cases were performed as part of the LMM and GEE analyses on each measure, and there were no significant differences (Tables S1–S5 in Supplement 1).

Discussion

We performed detailed diagnostic, cognitive, and behavioral testing, including standardized ASD assessment, on individuals who were ascertained after clinical identification of the 16p11.2 deletion and family member cascade testing and compared them with familial control subjects. Our protocol addressed challenges in prior studies by way of standardization and comprehensive phenotyping. Our analyses clearly indicate that individuals with the deletion have a high frequency and range of psychiatric and developmental disorders compared with noncarrier control subjects. The most commonly observed diagnoses were developmental coordination disorder, phonological processing disorder, language disorders, and ASD. One or more speech and language diagnoses were present in 71% of all individuals with 16p11.2 deletion, highlighting the specific contribution of this CNV to language development. There was significant psychiatric comorbidity; many individuals met criteria for multiple diagnoses (Figure S2). This diagnostic overlap and clustering provides avenues for further understanding of the phenotype of the 16p11.2 deletion.

Although 24% of all individuals with the 16p11.2 deletion had a diagnosis of ASD, most individuals with the deletion had significantly higher rates of autism-related characteristics, such as social and behavioral difficulties as reported on the SRS and repetitive and stereotyped behaviors as indexed by the BSIQ, compared with family members without the deletion. Children who did not meet criteria for any psychiatric diagnosis still had subthreshold challenges in the social communication and behavioral traits related to ASD. This finding highlights the quantitative effect of the 16p11.2 deletion on autism-related traits, even when full ASD diagnostic criteria are not met.

A novel finding we report in this sample is increased odds of left hand or mixed hand dominance in individuals with 16p11.2 deletion. This finding underscores potential differences in brain development and cerebral asymmetry and provides insight into the deficits observed.

Finally, individuals with the 16p11.2 deletion demonstrated varying levels of intellectual ability, and the average IQ was approximately 1 SD below the population mean. However, relative to family members without the deletion, participants with the deletion showed a 1.8 SD decrement in IQ. Together with the results of social and behavioral deficits, these IQ findings suggest that the 16p11.2 deletion, regardless of psychiatric diagnosis, broadly affects several aspects of brain development and function, including language, cognition, and social cognition.

Previous studies of the 16p11.2 deletion with smaller sample sizes have reported wide-ranging phenotypes, including cognitive impairments, language deficits, ASD, and behavioral problems (3,6). The findings in this large series, using a standardized assessment battery and experienced clinicians trained to reliability, confirm and expand on these previous findings compared with familial control subjects. We report similar findings of commonly observed language impairments, presence of a subgroup with ASD, and consistent cognitive impairments. Additionally, these results highlight the presence of articulation challenges, language disorders, and motor impairments in a significant minority of participants with a deletion as well as left hand and mixed hand dominance.

When these findings are compared against other clinically ascertained individuals with other deleterious CNVs, the uniqueness of the articulation, language, and motor impairments in the 16p11.2 deletion is apparent. That is, many similarly ascertained clinical patients with recurrent CNVs also share an effect on IQ and phenotypic variability but lack the specificity of language-based and motor-based impairments in the 16p11.2 deletion. For example, about 25% of clinical patients with 22q11.2 deletions (DiGeorge or velocardiofacial syndrome) have a psychiatric disturbance, such as ASD, ADHD, or schizophrenia (51). Clinically ascertained patients with 1q21.1 deletions have similar psychiatric phenotypic variability, including intellectual disability, ASD, and schizophrenia (52). However, neither the 22q11.2 deletion nor the 1q21.1 deletion shares the constellation of articulation, language, and motor impairments. Language, articulation, and motor challenges are common in ASD (53–58), underscoring the relevance of this locus to ASD specifically.

The composition of our sample provides insight into the 16p11.2 deletion phenotype. Despite efforts for broad inclusion of all individuals with the 16p11.2 deletion, our sample contains only seven adults with the deletion. It is essential to consider the ascertainment approach and study design employed in this study in light of these findings. Participants were recruited through the Simons VIP Connect website to which they were referred via medical genetics clinics, genetic counselors, and Internet searches and had been clinically diagnosed with a 16p11.2 deletion owing to clinical concerns that led to ordering a chromosome microarray. Participants completed a screening process and traveled (often a great distance) to the clinical testing site. All expenses were paid to remove financial barriers. When a patient was identified in a family, cascade genetic testing identified other family members with the deletion. This design led to self-selection of parents who could navigate the research recruitment and screening process as well as navigate travel with family members with a deletion. The small proportion of adults in the sample is likely a result of the challenges associated with study participation that preclude participation of adults with the deletion unless another adult (often a spouse) could navigate this system and coordinate participation. Several participants in the sample with inherited deletions were adopted, with records reporting behavioral challenges in the biological parent with the deletion that would have precluded caring for a child or participation in the study. In addition, there is an ascertainment bias against clinically asymptomatic individuals; such individuals would not have known they carried the deletion because most asymptomatic individuals have not had a chromosome microarray. Finally, the sample also contains far fewer individuals with inherited compared with de novo events, suggesting the possibility that the 16p11.2 deletion affects reproductive fitness, reducing the likelihood that this CNV will be transmitted.

Although our conclusions are potentially constrained by the above-described possible ascertainment biases, more recent work from a large study in Iceland (21) suggests that our findings may be broadly representative of most carriers of the CNV. As part of a larger study on CNVs, the researchers identified all carriers of the 16p11.2 deletion (n = 43) in a population sample of 101,655 Icelanders, representing roughly one third of the entire population of Iceland. The investigators administered an abbreviated cognitive battery to seven carriers of the CNV who did not have a psychiatric diagnosis. These carriers showed marked impairments in verbal and performance IQ as well as measures of verbal fluency and other cognitive domains, suggesting that our related observations in the Simons VIP sample are not an artifact of
clinical ascertainment. The Icelandic sample also sheds light on the small number of individuals with inherited deletions because fecundity in carriers was sharply reduced relative to other CNV carriers or the population at large; this likely explains the reduced likelihood that the CNV would be transmitted.

One study limitation is the potential increased noise resulting from the concatenation of data from multiple sites. The use of three sites employing experienced clinicians provided the ability to work with large numbers of individuals in a short amount of time. Examination of site influence on assessments revealed no differences between sites relative to the quantitative measures, suggesting that participants across sites were similar in cognitive, adaptive, social, language, and behavioral functioning. However, differences in diagnostic assignment across the sites in three psychiatric diagnoses were noted, suggesting the possibility of differences in diagnostic practices. Given the similarity of sample sizes at each site and the overall rates for these three diagnoses, although introducing additional noise, our findings indicate the robustness of the diagnostic picture of the 16p11.2 deletion and provide a reasonable estimate of the frequency with which each diagnosis is observed in clinical practice in which clinicians may apply different diagnostic labels.

These results have clinical implications. First, the consistent finding of a spoken-language deficit encompassing both receptive and expressive language as well as articulation highlights the importance of identifying and addressing communication challenges early in development. Second, given the high frequency of ASD and presence of the broader autism phenotype, careful consideration of autism-related symptoms is essential in any diagnostic characterization as well as in treatment planning. Subdiagnostic challenges in ASD-related domains can potentially moderate treatment outcome and adherence. Finally, motor coordination problems were found in >50% of our deletion carriers. Increased clumsiness, motor delays, and fine and gross motor coordination have not been consistently noted in the literature. However, given the high prevalence of motor impairment in our sample, it should be carefully assessed, and appropriate physical or occupational therapy should be initiated.

In conclusion, our analyses using data from a large, well-characterized series of individuals with the 16p11.2 deletion revealed a consistent, quantitative detrimental effect on cognition, language ability, and motor coordination; increased rates of ASD and the broader ASD phenotype (social difficulties, communication difficulties, stereotyped and repetitive behaviors and interests); and increased psychiatric difficulties compared with familial and population norms.

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