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Language Profiles of School-Age Children With 16p11.2 Copy Number Variants in a Clinically Ascertained Cohort

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ABSTRACT

Purpose: Individuals with proximal 16p11.2 copy number variants (CNVs), either deletions (16p11.2DS) or duplications (16p11.2Dup), are predisposed to neurodevelopmental difficulties and disorders, such as language disorders, intellectual disability, and autism spectrum disorder. The purpose of the current study was to characterize language profiles of school-age children with proximal 16p11.2 CNVs, in relation to the normative sample and unaffected siblings of children with 16p11.2DS.
Method: Standardized language tests were conducted in 33 school-age children with BP4–BP5 16p11.2 CNVs and eight unaffected siblings of children with 16p11.2DS to evaluate language production and comprehension skills across various language domains. A standardized intelligence test was also administered, and parents completed a standardized questionnaire to assess autistic traits. Language profiles were compared across 16p11.2 CNVs and intrafamilial pairs. The influence of nonverbal intelligence and autistic traits on language outcomes was investigated.
Results: No significant differences were found between children with 16p11.2DS and those with 16p11.2Dup, although both groups exhibited significantly poorer language skills compared to the normative sample and unaffected siblings of children with 16p11.2DS. Severe language deficits were identified in 70% of individuals with 16p11.2 CNVs across all language subdomains, with significantly better receptive vocabulary skills than overall receptive language abilities. In children with 16p11.2DS, expressive language deficits were more pronounced than receptive deficits. In contrast, only in children with 16p11.2Dup did nonverbal intelligence influence their language outcomes.
Conclusions: The current study contributes to the deeper understanding of language profiles in 16p11.2 CNVs in a clinically ascertained cohort, indicating generalized deficits across multiple language domains, rather than a syndrome-specific pattern targeting specific subdomains. The findings underscore the importance of early diagnosis, targeted therapy, and monitoring of language skills in children with 16p11.2 CNVs.
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Copy number variants (CNVs) between Breakpoints 4 and 5 (BP4–BP5) on chromosomal region 16p11.2, defined as proximal 16p11.2 deletion (16p11.2DS) and 16p11.2 duplication (16p11.2Dup) syndrome, predispose to neurodevelopmental disorders (NDDs), including language disorders (LDs), autism spectrum disorder (ASD), and intellectual disability (ID). 16p11.2DS primarily arises de novo (60%–76%), whereas 16p11.2Dup is more frequently inherited from a parent (71%–84%; Niarchou et al., 2019). Despite their association with a myriad of potential medical, cognitive, and behavioral symptoms, detailed data on language profiles in these CNVs remain limited (Chawner et al., 2021; Chung et al., 2021;

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Deshpande & Weiss, 2018; Green Snyder et al., 2016; Hanson et al., 2015; Moreno-De-Luca et al., 2015; Oliva-Teles et al., 2020; Rein & Yan, 2020; Steinman et al., 2016; Taylor et al., 1993, 2023). Both 16p11.2 CNVs are characterized by incomplete penetrance and variable expressivity (Kingdom & Wright, 2022; Verbesselt et al., 2024).

Language deficits associated with 16p11.2DS have been reported in several studies, revealing a spectrum of impairments across various language domains (Berman et al., 2015; Bijlsma et al., 2009; Chung et al., 2021; Deshpande & Weiss, 2018; Fedorenko et al., 2016; Hanson et al., 2015; Jiménez-Romero et al., 2022; Maillard et al., 2015; Matsuzaki et al., 2020; Mei et al., 2018; Rosenfeld et al., 2010; Shinawi et al., 2010). LDs are frequently observed in 16p11.2DS, with prevalence rates ranging from 42% to 83%, including expressive and mixed receptive–expressive LDs (Chung et al., 2021; Hanson et al., 2015; Mei et al., 2018). Mei et al. (2018) found average language scores of 2.1 *SD*s below the population mean in children with 16p11.2DS, whereas other studies reported a downward shift of 1.5–1.9 *SD*s (Ahtam et al., 2019; Berman et al., 2015; Blackmon et al., 2018; Hanson et al., 2015; Matsuzaki et al., 2020). Mei et al. identified core language deficits, affecting morphological, syntactic, and semantic language domains, in 83% (33/40) of children with 16p11.2DS, with language scores nearly 1 *SD* below their nonverbal cognitive skills. Based on direct language instruments, syntactic difficulties were prevalent in 78% of individuals with 16p11.2DS (Kim et al., 2020). Although both expressive and receptive language skills were impaired (Hanson et al., 2015; Mei et al., 2018), some studies noted a slight predominance of receptive over expressive language abilities (Ahtam et al., 2019; Blackmon et al., 2018; Hanson et al., 2010; Jiménez-Romero et al., 2022). School-age children with 16p11.2DS had significantly poorer language skills compared to their unaffected siblings (Hanson et al., 2015) and to typically developing controls (Ahtam et al., 2019; Berman et al., 2015).

Studies on language profiles in 16p11.2Dup are even more scarce and are mainly based on participants from the Simons Searchlight (Simons VIP Consortium, 2012). Children with 16p11.2Dup have language scores of 1.1–1.2 *SD*s below the population mean (Blackmon et al., 2018; Matsuzaki et al., 2020), with syntactic difficulties detected in 41%–46% of cases. Interestingly, their language scores were not significantly different from unaffected intrafamilial controls (Hippolyte et al., 2016).

Cross-CNV comparisons (16p11.2DS–16p11.2Dup) revealed contradictory findings regarding language differences. Most studies have reported no significant differences in language between 16p11.2DS and 16p11.2Dup (Blackmon et al., 2018; Matsuzaki et al., 2020), whereas others found significantly better language skills in children with 16p11.2Dup, but only in specific domains such as syntax and certain phonological skills (Hippolyte et al., 2016; Kim et al., 2020). Despite the significant impact of nonverbal cognitive skills on structural language outcomes in both CNV groups, language deficits remained present, even after accounting for ASD diagnosis and cognitive impairments (Kim et al., 2020).

Previous studies investigating language skills in individuals with 16p11.2 CNVs have primarily relied on data from the Simons VIP cohort or have lacked appropriate comparison groups. Although some research has examined correlations between language measures and brain structures or functions using neuroimaging studies, these efforts did not primarily focus on characterizing language profiles (Ahtam et al., 2019; Berman et al., 2016; Blackmon et al., 2018; Hippolyte et al., 2016; Matsuzaki et al., 2020). To address these gaps, the current study aims to conduct a comprehensive examination of language profiles in individuals with 16p11.2 CNVs from a Belgian cohort, focusing on comparisons with the population mean and with unaffected siblings of children with 16p11.2DS. Furthermore, we aim to compare language profiles across 16p11.2 CNVs to explore whether CNVs occurring within the same chromosomal region yield comparable phenotypic effects or if alterations in gene dosage correlate with mirrored phenotypes (Jacquemont et al., 2011). Finally, we aim to investigate whether confounding factors such as autistic traits and nonverbal cognitive skills have an influence on language outcomes.

Method

Participants

A cohort of 41 school-age children was included in the current study (M = 10;11 [years;months], SD = 3;1, range: 5;10–16;11). This group consisted of 23 unrelated children with proximal 16p11.2DS (BP4–BP5), 10 unrelated children with proximal 16p11.2Dup (BP4–BP5), and eight full-biological unaffected siblings of the children with 16p11.2DS. Only individuals whose first language was Dutch or with at least 3 years of full-time Dutch education were included (Cummins, 2000; Kohnert et al., 2021). Additional exclusion criteria were extreme prematurity (i.e., gestational age < 32 weeks), moderate-to-severe hearing impairment (≥35 dB HL; Barre et al., 2011; Crosbie et al., 2011; Lieu et al., 2020), distal CNVs outside the BP4–BP5 region, and additional pathogenic chromosomal variants. All 16p11.2 CNVs were confirmed using chromosomal microarray (CMA). The

sibling cohort consisted of siblings of children with a de novo 16p11.2DS. No CMA testing was done in these siblings (n = 7), except for one sibling who was confirmed not to have a pathogenic CNV on 16p11.2 (n = 1). Only eight children with 16p11.2DS had at least one sibling who met the criteria of age, being born at term and without neurological problems. When families had more than one eligible sibling, the one closest in age to the child with the 16p11.2DS was included.

Sociodemographic features for both CNV groups and unaffected siblings of children with 16p11.2DS are shown in Table 1. Speech-language therapy was received by all children with 16p11.2Dup, 78% of children with 16p11.2DS, and 12% of siblings. The majority of children with 16p11.2DS (83%) and 16p11.2Dup (80%) follow special education, whereas almost all siblings (7/8, 88%) attend regular education.

Procedure and Measurements

All individuals were included through the Centre for Human Genetics of University Hospitals Leuven or Maastricht University Medical Center. Using a standardized protocol, prospective data were collected from all participants both in the clinic and at home. The research protocol involved an interview on developmental language

Table 1. Sociodemographic characteristics across 16p11.2 copy number variants and siblings.

Variable	16p11.2Dup	16p11.2DS	Siblings of 16p11.2DS
Sample size (n)	10	23	8
Sex, n (%)			
Male	5 (50)	9 (39)	5/8 (62)
Female	5 (50)	14 (61)	3/8 (38)
Chronological age (years;months)			
Average (SD)	10.1 (2.8)	11.2 (3.2)	11.1 (3.6)
Median	9.5	10.1	10.5
Range	6;3–13;8	5;10–16;11	6;9–15;9
Type of education, n (%)			
Special education	8/10 (80)	19/23 (83)	1/8 (12)
Regular education	2/10 (20)	1/23 (4)	7/8 (88)
Regular with assistance	0/10 (0)	3/23 (13)	0/8 (0)
SES[a]			
High	3/10 (30)	9/23 (39)	4/8 (50)
Middle	5/10 (50)	12/23 (52)	3/8 (38)
Low	3/10 (30)	2/23 (9)	0/8 (0)
Unknown	0/10 (0)	0/23 (0)	1/8 (12)
Speech-language delays, n (%)	10/10 (100)	21/23 (91)	1/8 (12)
Speech-language therapy, n (%)	10/10 (100)	18/23 (78)	1/8 (12)
Mild hearing loss	1/10 (10)	7/23 (30)	0/8 (0)
Formal NDD diagnoses, n (%)			
ID (FSIQ < 70)	6/10 (60)	10/23 (43)	0/8 (0)
ASD	7/10 (70)	10/22 (45)	1/8 (12)
ADHD	6/10 (60)	7/22 (32)	1/8 (12)
Inheritance pattern, n (%)			
De novo	2/10 (20)	12/23 (52)	
Inherited	2/10 (20)	3/23 (13)	
Maternally inherited	2/2 (100)	2/3 (67)	
Paternally inherited	0/2 (0)	1/3 (33)	
Unknown[b]	6/10 (60)	8/23 (35)	

Note. SES = socioeconomic status; NDD = neurodevelopmental disorder; ID = intellectual disability; FSIQ = Full-Scale IQ; ASD = autism spectrum disorder; ADHD = attention-deficit/hyperactivity disorder.

[a]Educational attainment of the mother was used as a proxy for SES. The classification of SES was based on the International Standard Classification of Education of UNESCO (Organisation for Economic Co-operation and Development, 2017; UNESCO Institute for Statistics, 2012) using three categories: low (primary education or lower grades of high school), middle (secondary/high school graduate), and high (bachelor, master's, or doctoral degree). [b]Adopted (n = 1) and foster care (n = 2); maternal inheritance was ruled out, and parents declined genetic testing.

milestones (first words, use of two-word sentences), two standardized language tests, an intelligence test, and a standardized questionnaire on social responsiveness completed by parents or guardians.

Language Assessment

Language skills of participants were assessed using the Dutch versions of the Peabody Picture Vocabulary Test–Third Edition (PPVT-III-NL; Dunn & Dunn, 1997; Schlichting, 2005) and the Clinical Evaluation of Language Fundamentals–Fourth Edition (CELF-4-NL; Kort et al., 2010; Semel et al., 2010) or the Clinical Evaluation of Language Fundamentals Preschool–Second Edition (CELF-P2-NL; de Jong, 2012; Semel et al., 2004), depending on the age of the participant. The PPVT-III-NL was administered to evaluate receptive vocabulary, resulting in a word comprehension score (WCS) based on the chronological age (CA) of the child (M = 100, SD = 15; Dunn & Dunn, 1997; Schlichting, 2005). The CELF tests were used to evaluate both language comprehension and production abilities across various language subdomains, such as morphological, syntactic, and semantic skills. These instruments are commonly employed in clinical settings to diagnose individuals with LDs, define therapy objectives, and track their development over time. Furthermore, the CELF tests provide normative data for children aged 3–6 years on the CELF-P2 and 5–18 years on the CELF-4. Raw scores from each subtest were converted into scaled scores (SS) based on the child's CA (M = 10, SD = 3). SS falling within the range of 7–13 were categorized as average. SS of ≤ 6 indicated mild–moderate language difficulties, whereas SS of ≤ 3 indicated severe language deficits. The following receptive language subtests were administered: Word Classes-Receptive, Sentence Structure (SST; 5;00–8;11 years) or Semantic Relationships (SR; ≥ 9;00 years), and Concepts and Following Directions (5;00–12;11 years). The following expressive language subtests were administered: Word Classes-Expressive (WC-E), Formulated Sentences, Recalling Sentences (RS), Word Structure (5;00–8;11 years), Expressive Vocabulary (5;00–9;11 years), and Word Definitions (WD; ≥ 10;00 years). Scores of different receptive and expressive subtests were combined to derive the core, or composite, language scores (CLSs) as well as receptive (RLI) and expressive language index (ELI) scores depending on the CA (M = 100, SD = 15; age ranges: 5;00–8;11, 9;00–12;11, ≥ 13;00) and test versions (CELF-P2 vs. CELF-4). Nevertheless, the CLS represents an assessment of overall language proficiency for all ages and versions. Clinical thresholds were set at 85 (16th percentile, −1 SD) for mild language issues, 77 (6th percentile, −1.5 SD) for moderate language difficulties, and 70 (2nd percentile, −2 SD) for severe language deficits. As one participant's verbal skills were insufficient, only composite scores were used in the analyses, and subtest scores were not evaluated.

Cognitive Assessment

All participants were evaluated using the latest Dutch adaptation of the Wechsler Intelligence Scale for Children–Fifth Edition (Hendriks et al., 2019; Wechsler, 2014). Three children were excluded from retesting as they had undergone assessments within the previous year using the Dutch versions of the Wechsler Nonverbal Scale of Ability (Wechsler & Naglieri, 2008), Snijders–Oomen Nonverbal Intelligence Test–Revised (Tellegen & Laros, 2017), and Wechsler Preschool and Primary Scale of Intelligence–Fourth Edition (Wechsler, 2012). Full-Scale IQ (FSIQ) and the Nonverbal Index (NVI) were calculated for all participants based on age-referenced norm tables (M = 100, SD = 15).

Social Responsiveness Assessment

Parents or caregivers completed the Dutch version of the Social Responsiveness Scale–Second Edition (SRS-2; Constantino & Gruber, 2012; Roeyers et al., 2015). The SRS-2 is a screening tool consisting of 65 questions divided in five treatment scales to assess autistic traits. Using the sex- and country-normed tables for children aged 4–18 years, a total t score is calculated (M = 50, SD = 10), with higher scores indicating more pronounced social issues. Total t scores falling within the range of 61–75 (percentile: 1.2–16) suggest mild–moderate impairments, whereas t scores exceeding 75 (percentile < 1.2) indicate severe deficits in social responsiveness.

Statistical Analyses

Our study design involved a prospective cross-sectional approach, integrating categorical and dimensional perspectives, alongside independent and pairwise comparisons. We set a significance threshold of $p < .05$, with Benjamini–Hochberg false discovery rates applied to address potential type I errors from multiple comparisons (Benjamini & Hochberg, 1995; Benjamini & Yekutieli, 2001). Adjusted p values fell within the range of .0071–.05. Additionally, we computed confidence intervals at the 95% level for all outcome variables. Statistical analyses were performed using R 4.2.1 (R Core Team, 2017; Wickham, 2016) and JASP Version 0.16.3 (JASP Team, 2022).

Initially, we assessed whether language, cognitive, and social responsiveness abilities of children with 16p11.2 CNVs and unaffected siblings of children with 16p11.2DS deviated from those of the normative sample. Depending on the violation of assumptions, this was analyzed using either Student's or Wilcoxon signed-ranks one-sample

t tests. Subsequently, we conducted cross-CNV and intrafamilial comparisons. Given the expected large within-group variability for both CNVs, traditional statistical tests were combined with descriptive statistics in a three-tiered research approach. These comparisons were conducted across three distinct levels to attain a comprehensive understanding of the language differences: (a) statistical tests on a group level, (b) analysis of percentage differences within and across subgroups, and (c) identification of (un)expected individual trends in the data (Olsson, 2005).

At the group level, cross-CNV comparisons were executed through independent Student's t tests or Mann–Whitney U tests for seven composite scores (PPVT: WCS, CELF: CLS/ELI/RLI, Wechsler scales: FSIQ/NVI, SRS-2 total t score), with Cohen's d or rank biserial correlation r as the effect size. Intrafamilial comparisons were conducted using paired-samples Student's t tests for the same six composite scores, with Cohen's d as the effect size. Differences between composite scores were calculated for each group for three pairwise comparisons (ELI vs. RLI, WCS vs. RLI, and CLS vs. NVI) using paired-samples Student's or Wilcoxon signed-ranks t tests. At the subgroup level, we calculated the percentages of children with 16p11.2 CNVs and siblings exhibiting mild–moderate to severe issues, based on the appropriate cutoff scores for each test (PPVT/CELF/Wechsler scales < 85). We determined differences in proportions through the Fisher's exact test, with odds ratio serving as the effect size measure. Discrepancies between composite scores were defined as a difference of at least 15 points between both summary scores, as differences of at least 1 SD are only seen in 14%–16% of the normal population and can be interpreted as clinically relevant (Semel et al., 2010). For CELF subtests, children were identified as experiencing language difficulties if their scores deviated by more than 1 SD from the population mean (SS < 7). At the individual level, we examined the effects of nonverbal skills (NVI) and autistic traits (SRS-2 total t scores) on the CLSs, using linear regression models for each CNV separately with SRS-2 and NVI as covariates. Like Kim et al. (2020), we also investigated significant impacts of the intercepts in the regression model to ascertain if the anticipated language score for a participant with average NVI and SRS is significantly lower than the population mean. Therefore, we transformed the SS into z scores ($z = \frac{SS - Average_{population}}{SD_{population}}$). Other potential confounding factors, such as sex, inheritance pattern, comorbid ASD and attention-deficit/hyperactivity disorder (ADHD), and socioeconomic status (SES), were explored through descriptive statistics and/or independent t tests. Venn diagrams were plotted to visualize the number of children with severely affected (< 70) (a) RLI, ELI, and/or NVI and (b) CLS, SRS, and/or NVI.

Results

Language and IQ in 16p11.2 CNVs and Siblings Compared to the Norm Group

Figure 1 depicts the box plots of the six summary scores for the three groups of children. Heterogeneous profiles, as indicated by the broad range of scores, are seen in the three groups of children, but predominantly in the 16p11.2Dup group. The descriptive statistics for these variables are summarized in Table 2.

The density plots for the CELF CLSs are displayed in Figure 2. The dashed line refers to the normal distribution of the normative sample (M = 100, SD = 15). In relation to the norm group, the distributions of children with 16p11.2DS and children with 16p11.2Dup demonstrate a downward shift of 2.33 SDs (≈ 25 CLS points) and 2.36 SDs (≈ 26 CLS points), respectively, whereas the distribution of unaffected siblings of children with 16p11.2DS shows a downward shift of 0.49 SD (≈ 7 CLS points). The distributions of children with 16p11.2 CNVs are considerably overlapping. We performed one-sample t tests, revealing statistically significantly lower scores in 16p11.2DS (p < .001) and 16p11.2Dup (.001 > p > .010) for all composite scores with large effect sizes (d < −1.036, r < −.993; see Supplemental Material S1). The scores of the siblings of children with 16p11.2DS did not significantly differ from the norm group scores (.081 < p < .200).

Cross-CNV and Intrafamilial Comparisons at Group Level: Mean Differences

In both CNV groups, average CELF CLSs fell within the severe range (< 70), whereas PPVT WCSs were within the mild–moderate range (70–84). Children with 16p11.2DS had intelligence scores in the borderline range (IQ, 70–84), whereas children with 16p11.2Dup were between borderline functioning and mild ID (< 70). Siblings' average scores were within the (low) average range (85–100). Social responsiveness scores were in the severe range for 16p11.2 CNVs, whereas siblings obtained average scores. Group-level analyses revealed no significant differences in any of the seven composite scores between children with 16p11.2DS and those with 16p11.2Dup (p > .209; see Table 2). However, children with 16p11.2DS demonstrated statistically significantly lower composite scores than their unaffected siblings (.001 < p < .047).

Children with 16p11.2DS demonstrated significantly lower ELI scores (M = 63.61) compared to RLI scores (M = 71.91; p < .001, d = 0.818; see Supplemental Material S2), with a large effect size. Conversely, their siblings exhibited comparable RLI and ELI scores (ELI M = 90.75, RLI M = 92.88; p = .469, d = 0.271) as did

Figure 1. Box plots for CELF, PPVT, and IQ scores across 16p11.2 copy number variants and siblings of those with 16p11.2DS. The dashed lines illustrate the norm group averages. The gray zones refer to the severity of the deficits; the darker the gray, the more severe the deficits: light gray zone = mild–moderate and darker gray zone = severe, based on clinical cutoff scores for CELF, PPVT, and Wechsler scales. CELF = Clinical Evaluation of Language Fundamentals; CLS = core language score; ELI = expressive language index; RLI = receptive language index; PPVT = Peabody Picture Vocabulary Test; WCS = word comprehension score; FSIQ = Full-Scale IQ; NVI = Nonverbal Index (average = 100, *SD* = 15 in the normative sample; cutoff: < 85 = mild–moderate, < 70 = severe); SRS-2 = Social Responsiveness Scale–Second Edition (average = 50, *SD* = 10 in the normative sample; cutoff: > 60 = mild–moderate, > 75 = severe).

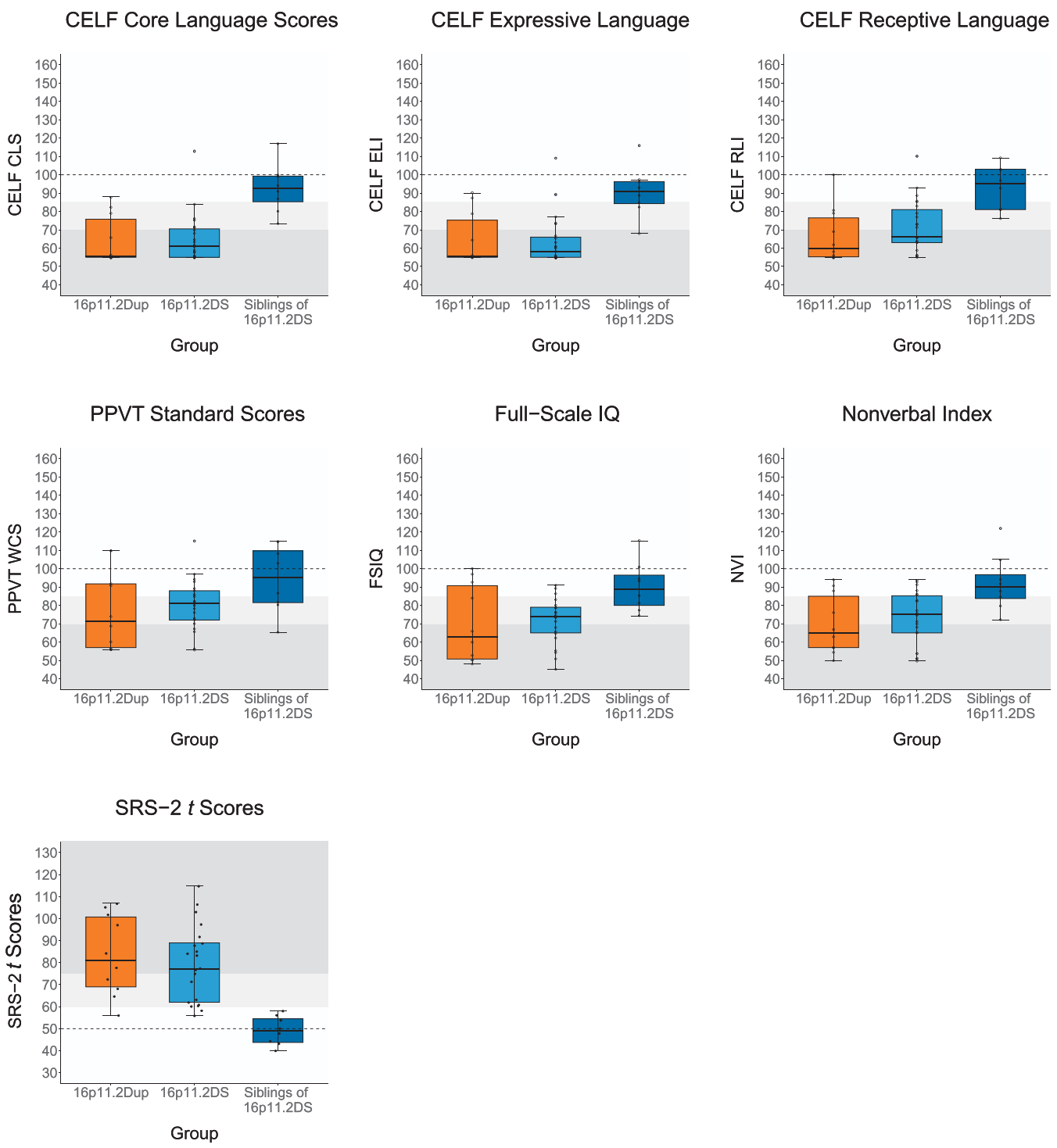


Table 2. Cross-copy number variant and intrafamilial comparisons across Clinical Evaluation of Language Fundamentals (CELF) composite, Peabody Picture Vocabulary Test (PPVT), and IQ scores.

Measure	16p11.2DS (*n* = 23)	16p11.2Dup (*n* = 10)	Statistical outcomes independent *t* test	16p11.2DS (*n* = 8)	Siblings of 16p11.2DS (*n* = 8)	Statistical outcomes paired *t* test
CELF CLS, *M* (*SD*)	65.04 (13.40)	64.00 (13.31)	*W* = 126.000	63.13 (7.95)	92.63 (13.47)	*t* = –6.123
Median	61	55.5	*p* = .673	63	92.5	***p* < .001****
Range	55.00–113.00	55.00–88.00	*r* = .096	55.00–76.00	73.00–117.00	*d* = –2.165
95% confidence interval	59.25, 70.84	55.08, 74.12		56.47, 69.77	81.36, 103.89	
CELF RLI, *M* (*SD*)	71.91 (14.09)	67.00 (15.25)	*W* = 147.500	71.88 (12.63)	92.88 (12.24)	*t* = –3.650
Median	66	60	*p* = .209	72	95	***p* = .008****
Range	55.00–110.00	55.00–100.00	*r* = .283	55.00–89.00	76.00–109.00	*d* = –1.290
95% confidence interval	65.82, 78.01	56.09, 77.91		61.31, 82.44	82.64, 103.11	
CELF ELI, *M* (*SD*)	63.61 (13.40)	65.10 (14.48)	*W* = 114.40	61.13 (6.90)	90.75 (13.83)	*t* = –6.922
Median	58	55.5	*p* = 1.000	59.5	91	***p* < .001****
Range	55.00–109.00	55.00–90.00	*r* = –.004	55.00–73.00	68.00–116.00	*d* = –2.447
95% confidence interval	57.81, 69.40	54.74, 75.46		55.36, 66.89	79.19, 102.31	
PPVT WCS, *M* (*SD*)	80.29 (13.86)	77.40 (21.81)	*t* = 0.449	77.88 (14.34)	94.38 (18.47)	*t* = –2.400
Median	81	71.5	*p* = .657	76	95	***p* = .047****
Range	56.00–115.00	56.00–110.00	*d* = 0.143	56.00–97.00	65.00–115.00	*d* = –0.849
95% confidence interval	73.98, 86.59	61.80, 92.00		56.89, 89.86	78.93, 109.82	
FSIQ, *M* (*SD*)[a]	71.33 (12.55)	70.10 (21.20)	*W* = 113.50	69.43 (9.05)	90.13 (13.69)	*t* = –4.228
Median	74	63	*p* = .735	74	89	***p* = .006****
Range	45.00–91.00	48.00–100.00	*r* = .081	54.00–79.00	74.00–115.00	*d* = –1.598
95% confidence interval	65.62, 77.05	54.94, 85.26		61.06, 77.80	78.68, 101.57	
NVI, *M* (*SD*)	73.00 (14.35)	69.70 (16.43)	*t* = 0.569	70.38 (14.42)	92.25 (15.50)	*t* = –4.868
Median	75	65	*p* = .574	73.5	90	***p* = .002****
Range	50.00–94.00	50.00–94.00	*d* = 0.217	50.00–91.00	72.00–122.00	*d* = –1.721
95% confidence interval	66.59, 79.32	66.59, 79.32		58.32, 82.43	79.29, 105.21	
SRS, *M* (*SD*)	79.14 (17.32)	83.40 (18.38)	*t* = –0.628	84.13 (15.82)	49.13 (6.53)	*t* = 5.457
Median	77	81	*p* = .535	83.5	49	***p* < .001****
Range	56.00–115.00	56.00–107.00	*d* = –0.241	62.00–115.00	40.00–58.00	*d* = 1.929
95% confidence interval	71.26, 87.03	70.25, 96.55		70.90, 97.35	43.66, 54.59	

Note. CLS = core language score; RLI = receptive language index; ELI = expressive language index; WCS = word comprehension score; FSIQ = Full-Scale IQ; NVI = Nonverbal Index (norm group average = 100; cutoff: < 85: mild–moderate, < 70: severe); SRS = Social Responsiveness Scale (norm group average = 50; cutoff: > 60: mild–moderate, > 75: severe).

[a]One participant's verbal skills were insufficient, resulting in only NVI scores being available for analysis.

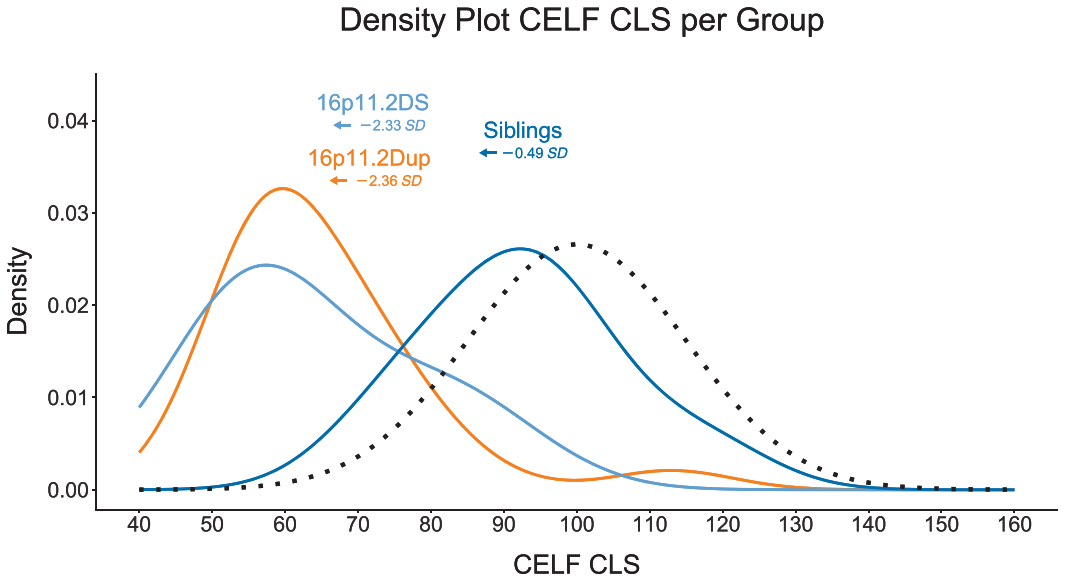
**Significant with false discovery rate; *t* value or *W* value; Cohen's *d* or rank biserial correlation *r* as effect size.

children with 16p11.2Dup (ELI M = 65.10, RLI M = 67.00; p = .218, d = 0.419). Receptive vocabulary (PPVT WCS) was statistically significantly higher than CELF RLI for both CNV groups with large effect sizes (DS: p = .001, Dup: p = .018, r = −.911), but not for siblings of children with 16p11.2DS (p = .731, d = −0.115). Similarly, CELF CLSs were statistically significantly lower than NVI in 16p11.2Dup (p = .016, d = −0.940) with a large effect size, but not in those with 16p11.2DS (p = .032, d = −0.491) or their siblings (p = .920, d = 0.037). Whereas siblings obtained low-average scores on all subtests, subtest scores for both CNVs fell in the mild–moderate to severe range with comparable distributions, indicating similar language difficulties among children with 16p11.2 CNVs (see Supplemental Materials S3 and S4). In the 16p11.2DS cohort, individuals who experienced delayed speech-language milestones during infancy (n = 21) exhibited an average CLS of 62.42, whereas those without speech-language delays (n = 2) had an average CLS of 92.50.

Cross-CNV and Intrafamilial Comparisons at Subgroup Level: Proportion Differences

Table 3 presents the percentages of individuals with mild–moderate to severe deficits across composite and subtest scores on the CELF and PPVT. Subgroup-level comparisons of the percentages did not reveal any significant differences between children with 16p11.2DS and children with 16p11.2Dup (p > .358). However, significantly more deficits were observed in children with 16p11.2DS compared to their unaffected siblings (.001 < p < .007), except for receptive

Figure 2. Normative distributions of CELF CLS for the three groups of children (16p11.2DS, 16p11.2Dup, and siblings of those with 16p11.2DS). The dashed line depicts the standard distribution of the normative sample (M = 100, SD = 15). Standard deviation changes are determined relative to typically developing peers in the general population, represented by the norm group. CELF = Clinical Evaluation of Language Fundamentals; CLS = core language score.



language skills (CELF RLI: p = .315, PPVT WCS: p = .619) and FSIQ (p = .026; see Supplemental Material S5).

Clinically relevant poorer expressive than receptive language skills, defined as a difference of at least 15 points between both summary scores, were found in six of 23 (26%) children with 16p11.2DS, none of those with 16p11.2Dup, and none of their siblings. Receptive vocabulary was significantly better (difference ≥ 15) than overall receptive language in five of 21 (21%) children with 16p11.2DS, two of 10 (20%) children with 16p11.2Dup, and one of eight (13%) siblings. The NVI was significantly higher than the overall language score (CLS) in nine of 22 (41%) children with 16p11.2DS, none of those with 16p11.2Dup, and none of the siblings.

Using appropriate cutoff scores, core language deficits were identified in 96% of children with 16p11.2DS and in 90% of those with 16p11.2Dup. The majority of both CNV groups (70%) exhibited severe language deficits (< −2 *SD*). In addition, the lowest CLS (SS of 55) was obtained by 30% (7/23) of children with 16p11.2DS and 50% (5/10) of children with 16p11.2Dup. The most common difficulties in children with 16p11.2DS were problems with WD in 92%, RS in 91%, and WC-E in 82%. In the 16p11.2Dup group, most deficits were impairments in WD and RS in all participants and SST/SR in 90%. Across all subtests, impaired subtest scores were observed in no more than two siblings. In the 16p11.2DS group, all children who had delayed speech-language milestones also demonstrated affected CLS (21/21). Among those with normal acquired language milestones, one child had average CLS, whereas the other displayed impaired CLS. Similarly, children with 16p11.2Dup who experienced speech-language delays also showed impaired CLS (9/10), except for one child.

Impact of Confounding Factors on Language Outcomes in 16p11.2 CNVs

Although linear regression models did not reveal a significant influence of NVI or SRS on CLS in 16p11.2DS (p = .524), a significant impact of NVI was found in 16p11.2Dup (p < .001) with a large coefficient of determination (R^2) of 92.3% (see Supplemental Material S6). Intercepts of the alternative models (H1) indicated that children with 16p11.2DS without NVI impairments or autistic traits would still demonstrate language scores of 1.8 *SD*s lower than expected based on norm group scores (p = .003), whereas children with 16p11.2Dup would exhibit language scores of 0.7 *SD* lower than expected (p = .011). Independent t tests did not reveal any significant differences between children with 16p11.2 CNVs with or without ASD (p > .410). Similarly, no differences were found between children with 16p11.2 CNVs with or without ADHD (p > .187) or between male and female children (p > .114). Descriptive statistics for these confounding factors and SES can be found in Supplemental Material S7.

Scatter plots of CELF CLS in function of NVI and SRS are displayed in Figures 3A and B. Figure 3C illustrates the comorbidity between expressive (ELI), receptive (RLI), and nonverbal (NVI) skills, based on clinical cutoffs for

Table 3. Proportions of children with difficulties across composite scores and subtest scores on Clinical Evaluation of Language Fundamentals (CELF) and Peabody Picture Vocabulary Test (PPVT).

Score		16p11.2Dup	16p11.2DS	Siblings of 16p11.2DS
Sample size (*n*)		10	23	8
Composite scores	PPVT WCS (< –1 *SD* and < –2 *SD*)	6/10 (60%)	13/21 (62%)	3/8 (38%)
	Mild–moderate (< –1 *SD*)	1/10 (10%)	9/21 (43%)	2/8 (25%)
	Severe (< –2 *SD*)	5/10 (50%)	4/21 (19%)	1/8 (13%)
	CELF CLS (< –1 *SD* and < –2 *SD*)	9/10 (90%)	22/23 (96%)	2/8 (25%)
	Mild–moderate (< –1 *SD*)	2/10 (20%)	6/23 (26%)	2/8 (25%)
	Severe (< –2 *SD*)	7/10 (70%)	16/23 (70%)	0/8 (0%)
	CELF RLI (< –1 *SD* and < –2 *SD*)	9/10 (90%)	18/23 (78%)	3/8 (38%)
	Mild–moderate (< –1 *SD*)	2/10 (20%)	6/23 (26%)	3/8 (38%)
	Severe (< –2 *SD*)	7/10 (70%)	12/23 (52%)	0/8 (0%)
	CELF ELI (< –1 *SD* and < –2 *SD*)	8/10 (80%)	21/23 (91%)	2/8 (25%)
	Mild–moderate (< –1 *SD*)	1/10 (10%)	3/23 (13%)	1/8 (12%)
	Severe (< –2 *SD*)	7/10 (70%)	18/23 (78%)	1/8 (13%)
Receptive subtest scores	CFD (5;00–12;11 years)	5/8[a] (62%)	10/13 (77%)	1/5 (20%)
	SST/SR	9/10 (90%)	15/22 (68%)	2/8 (25%)
	WC-R	6/10 (60%)	15/22 (68%)	1/8 (12%)
Expressive subtest scores	RS	10/10 (100%)	20/22 (91%)	2/8 (25%)
	FS	8/10 (80%)	16/20 (80%)	2/8 (25%)
	WC-E	7/10 (70%)	18/22 (82%)	1/8 (12%)
	WS (5;00–8;11 years)	3/4 (75%)	6/8 (75%)	1/2 (50%)
	EV (5;00–9;11 years)	3/6 (50%)	7/10 (70%)	1/4 (25%)
	WD (> 9;00 years)	4/4 (100%)	11/12 (92%)	1/4 (25%)
Combined receptive and expressive subtest scores	WC-T	7/10 (70%)	16/22 (73%)	1/8 (12%)

Note. WCS = word comprehension score; CLS = core language score; RLI = receptive language index; ELI = expressive language index (cutoff: < 85, mild–moderate; < 70, severe); CFD = Concepts and Following Directions; SST = Sentence Structure (5;00–8;11 years); SR = Semantic Relations (≥ 9;00 years); WC-R = Word Classes-Receptive; RS = Recalling Sentences; FS = Formulated Sentences; WC-E = Word Classes-Expressive; WS = Word Structure; EV= Expressive Vocabulary; WD = Word Definitions (cutoff: < 7: mild–moderate problems; < 4: severe problems); WC-T = Word Classes-Total.

[a]Available data vary by subtest due to different age ranges of specific subtests or missing data.

severe deficits across the three variables (< 70). Clinical scores on these three variables were ascertained in 30% (7/22) of children with 16p11.2DS and 60% (6/10) of those with 16p11.2Dup. This figure also shows that severe language deficits mostly affected both language production and comprehension (48%, 11/23), followed by only productive (30%, 7/23) and only comprehensive (4%, 1/23) skills. Figure 3D shows the comorbidity between language (CELF CLS), nonverbal skills (NVI), and social responsiveness (SRS) based on the clinical cutoffs for the three variables. Severe deficits on the three variables were found in 23% (5/22) of children with 16p11.2DS and 50% (5/10) of those with 16p11.2Dup.

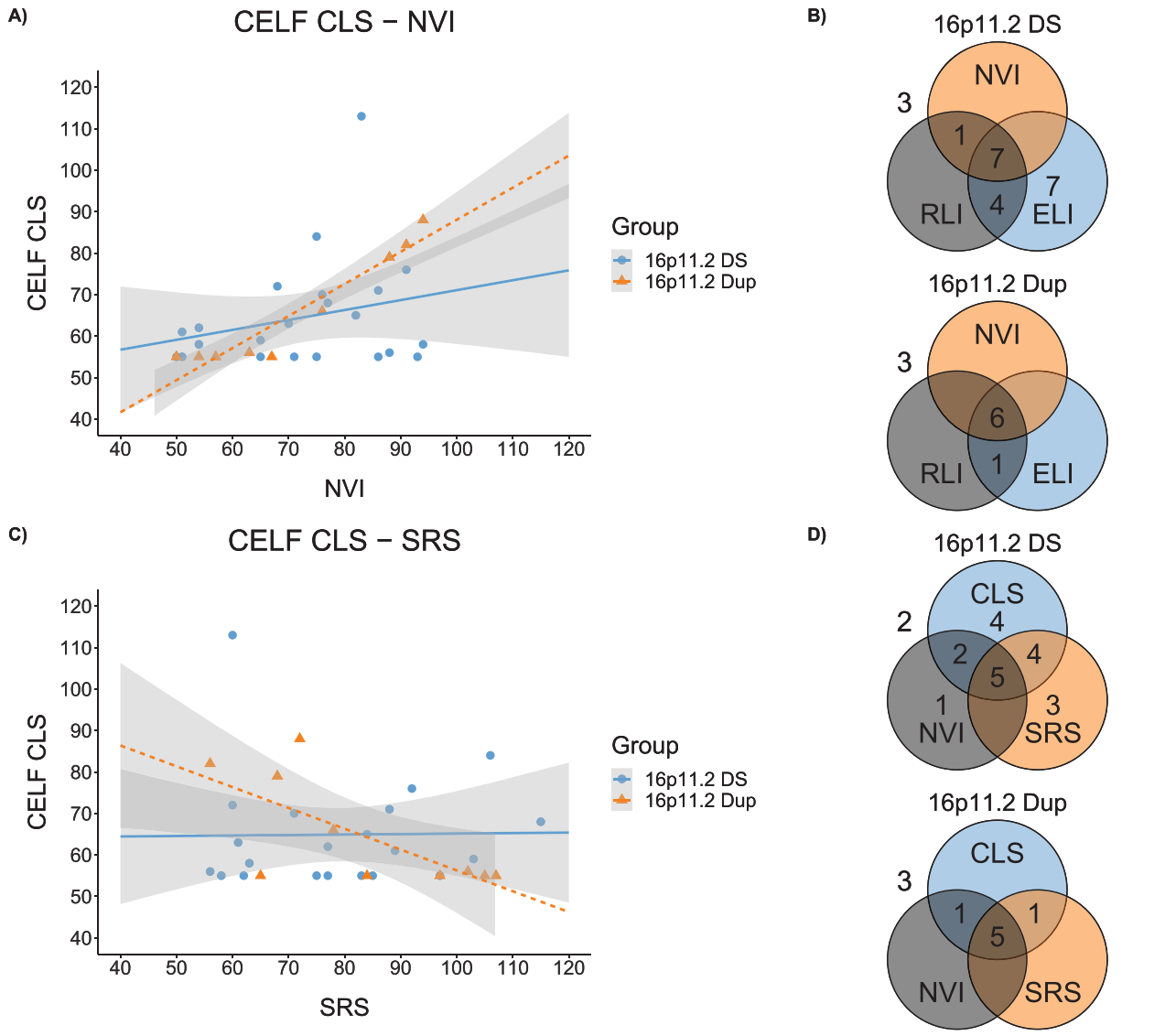
Discussion

The current study aimed to conduct a comprehensive examination of language profiles in individuals with 16p11.2 CNVs, focusing on cross-CNV and intrafamilial comparisons for 16p11.2DS and investigating the influence of the NVI and social responsiveness on language outcomes. We found no significant differences between children with 16p11.2DS and those with 16p11.2Dup, whereas both groups exhibited significantly poorer language skills compared to the normative sample and unaffected siblings of children with 16p11.2DS. Severe language deficits were identified in 70% of individuals with 16p11.2 CNVs across all language subdomains, with both groups exhibiting significantly better receptive vocabulary skills than overall receptive language abilities. Expressive language deficits were more pronounced than receptive deficits in children with 16p11.2DS. Nonverbal intelligence had an influence on language outcomes in children with 16p11.2Dup only.

16p11.2 CNVs and Siblings in Comparison to the Norm Group

Box plots revealed heterogeneous profiles, especially in the 16p11.2Dup group, aligning with prior studies that have shown broader phenotypic variability in 16p11.2Dup

Figure 3. (A) Scatter plot association between CELF CLS and NVI. (B) Scatter plot association between CELF CLS and SRS-2 total *t* score. (C) Comorbidity in 16p11.2 CNVs: participants with severe scores on NVI, RLI, and ELI. (D) Comorbidity in 16p11.2 CNVs: participants with severe scores on CLS, NVI, and SRS. The number outside the Venn diagram refers to the remaining participants for whom all three test scores were obtained. CELF = Clinical Evaluation of Language Fundamentals; CLS = core language score; CNVs = copy number variants; RLI = receptive language index; ELI = expressive language index; NVI = Nonverbal Index (cutoff: < 70, severe); SRS = Social Responsiveness Scale (cutoff: > 75, severe).



when compared to 16p11.2DS (Green Snyder et al., 2016). Children with 16p11.2 CNVs exhibited significantly poorer language skills compared to the normative sample, consistent with previous studies (Ahtam et al., 2019; Berman et al., 2015). For core language skills, we identified a shift of approximately −2.3 *SD*s in both CNV groups, which is more pronounced than reported in previous studies with shifts of −1.5 to 2.1 *SD*s in 16p11.2DS and of −1.1 to 1.2 *SD*s in 16p11.2Dup (Ahtam et al., 2019; Berman et al., 2015; Blackmon et al., 2018; Matsuzaki et al., 2020; Mei et al., 2018). Also for FSIQ, we detected a downward shift of approximately −2 *SD*s in both CNV groups, which is more pronounced than reported previously where FSIQ fell within the borderline range (IQ, 70–84; Blackmon et al., 2018; Chawner et al., 2021; D'Angelo et al., 2016; Gill et al., 2014; Green Snyder et al., 2016; Hanson et al., 2015; Hippolyte et al., 2016; Jutla et al., 2020; Kim et al., 2020; Maillard et al., 2016; Matsuzaki et al., 2020; Mei et al., 2018; Modenato et al., 2021; Moreau et al., 2020; Moreno-De-Luca et al., 2015; Owen et al., 2018; Taylor et al., 2023; Zufferey et al., 2012). These poorer language and IQ outcomes might be partially attributed to the clinical

ascertainment of this cohort. All participants received their CNV diagnosis based on either medical or developmental indications, which may bias the cohort toward individuals with more pronounced symptoms or impairments. Additionally, the inclusion criteria were restricted to index patients only, representing the severe end of the spectrum of our study population.

On the contrary, the language and cognitive skills of unaffected siblings of children with 16p11.2DS did not show a significant deviation from the normative sample, affirming their representativeness. Qualitatively, their scores were in the low-average range (−0.5 *SD*), which is similar to one study (Hippolyte et al., 2016), but lower compared to unaffected siblings and typically developing controls in other studies who obtained high-average scores (Ahtam et al., 2019; Berman et al., 2015; Hanson et al., 2015; Owen et al., 2018). This finding suggests that, in addition to the 16p11.2 CNVs, the broader familial and genetic background also plays a role in their cognitive and language outcomes, as was earlier suggested in 22q11.2 CNVs (Verbesselt, Van Den Heuvel, et al., 2022).

Language and IQ Differences Across 16p11.2DS and 16p11.2Dup

On average, both CNV groups demonstrated severe core language deficits, affecting both receptive and expressive language across morphological, syntactic, and semantic language domains, alongside mild–moderate deficits in receptive vocabulary. At the group level, none of the language composite scores differed significantly between both 16p11.2 CNVs. These findings are mostly consistent with previous research indicating comparable language skills across both CNV groups (Blackmon et al., 2018; Hippolyte et al., 2016; Kim et al., 2020; Matsuzaki et al., 2020). However, Kim et al. (2020) reported significantly better syntactic skills and expressive communication in children with 16p11.2Dup, whereas Hippolyte et al. (2016) identified better performance in this group for specific phonological skills, such as nonword repetition and oromotor sequences (Hippolyte et al., 2016; Kim et al., 2020). Furthermore, no significant differences were found for intelligence scores (FSIQ and NVI) between both groups, which is consistent with most previous findings (Blackmon et al., 2018; Gill et al., 2014; Hippolyte et al., 2016; Jutla et al., 2020; Maillard et al., 2015; Matsuzaki et al., 2020; Modenato et al., 2021; Moreau et al., 2020; Owen et al., 2018). However, two studies indicated that children with 16p11.2DS demonstrated significantly higher NVI or FSIQ than children with 16p11.2Dup (Chawner et al., 2021; Kim et al., 2020).

From a categorical perspective at subgroup level, core language deficits (< −1 *SD*, 16th percentile) were identified in 96% of children with 16p11.2DS and 90% of children with 16p11.2Dup, which is slightly more than in the study of Mei et al. (2018) for 16p11.2DS (33/40, 83%). The majority of both groups (7/10 and 16/23, 70%) experienced severe language deficits (< −2 *SD*, 2nd percentile). Similarly, no significant proportion differences were found at the subgroup level. Differences in language and intelligence outcomes across studies may arise from slightly different age ranges, used test instruments, and ascertainment strategies, as our clinical cohort predominantly represents the more severe end of the phenotypic spectrum. Future studies with larger samples should further unravel specific differences and similarities in language abilities across 16p11.2 CNVs.

In both groups, individuals with delayed speech-language milestones tended to exhibit impaired CLSs, highlighting the importance of early language milestones as predictors of later language outcomes. Therefore, systematic assessment of developmental milestones remains clinically relevant for identifying potential language delays and providing timely intervention.

Language and IQ Differences Between 16p11.2DS and Unaffected Siblings

At the group level, children with 16p11.2DS showed significantly lower composite scores than their unaffected siblings, indicating poorer language and cognitive outcomes, which is consistent with previous findings (Hanson et al., 2015). Compared to intrafamilial controls, Hippolyte et al. (2016) only found significant differences for specific subdomains, such as certain phonological and lexical skills. Similarly, at the subgroup level, children with 16p11.2DS exhibited significantly more deficits compared to their unaffected siblings for expressive and total language skills. The proportion differences for FSIQ and receptive language skills did not reach significance, probably due to the relatively small sample size and the fact that up to three (3/8) siblings showed mild–moderate deficits across these composite scores. These varying results in siblings underscore the importance of the interplay between genetic factors and environmental influences, such as the shared familial background, on neurodevelopmental outcomes.

Differences Across Composite Scores

Although both receptive and expressive language skills were impaired, this is the first study in which expressive language appears to be significantly more severely affected than receptive language in children with 16p11.2DS, with a receptive-over-expressive discrepancy (difference > 15) in 26% (6/23). This indicates that these children experience more difficulties with expressing

themselves verbally compared to understanding language. This finding was further supported by the observation that the three most prevalent difficulties in children with 16p11.2DS were related to expressive language subtests. In line with the current results, some studies noted a slight predominance of receptive over expressive language abilities in 16p11.2DS (Ahtam et al., 2019; Blackmon et al., 2018; Hanson et al., 2010; Jiménez-Romero et al., 2022), although both domains were affected in all studies (Hanson et al., 2015; Mei et al., 2018).

In contrast, children with 16p11.2Dup and unaffected siblings demonstrated comparable levels of receptive and expressive language skills, with their most frequent difficulties spanning both domains. Although one study reported better receptive than expressive language in children with 16p11.2Dup, it is important to note that this observation was based on a small sample size (n = 3 for RLI; Blackmon et al., 2018). Across both CNV groups, formulating word definitions and recalling sentences were the two most encountered challenges, indicating shared deficits across expressive semantics and syntax as well as auditory memory. Interestingly, the third most prevalent challenge in 16p11.2DS also related to expressive semantics, whereas it related to receptive syntax in those with 16p11.2Dup. These specific areas might warrant focused attention in speech-language therapy. However, it is important to acknowledge that all subtests were impacted in at least half of the children. Furthermore, caution is needed in overinterpreting the findings due to variations in sample sizes across subtests given the specific age ranges (e.g., WD $\geq$ 10;00 years, only administered in four children with 16p11.2Dup and 12 with 16p11.2DS).

Despite the receptive–expressive discrepancy in a subset of children with 16p11.2DS, a relatively consistent pattern emerged for both CNV groups, indicating both receptive and expressive language deficits in most children. Moreover, it is worth noting that the language deficits are not limited to a specific language aspect; instead, they broadly affect different language domains, including both lexico-semantic (i.e., language content) and morphosyntactic (i.e., language form) skills, in line with previous research (Mei et al., 2018), but not supporting the results in one boy with a smaller 16p11.2DS (Jiménez-Romero et al., 2022). This pattern of no relative strengths and weaknesses is also observed in other rare genetic disorders linked with cognitive impairments, such as Kabuki syndrome, NRXN1 deletions, Koolen de Vries syndrome, and Floating–Harbor syndrome (Brignell et al., 2018; Morgan et al., 2015, 2018; White et al., 2010).

The second comparison of composite scores revealed significantly stronger receptive vocabulary compared to overall receptive skills in both CNV groups, but not in siblings. Overall receptive skills refer to the ability to link two related words, the comprehension of sentence structures, and following oral directions. Therefore, this finding might suggest that while word-level receptive skills remain relatively intact, challenges may arise predominantly at the sentence level, which was also noted in children with 22q11.2Dup (Verbesselt et al., 2023). As almost all children attend special education, an additional explanation for difficulties with oral directions could be that task-specific concepts such as "the first, in between, before, after" have not been introduced or practiced yet in special education settings.

The third comparison between core language and nonverbal cognitive skills indicated poorer language than cognitive skills in 16p11.2Dup but not in 16p11.2DS or their siblings. However, in 41% (9/22) of children with 16p11.2DS, language scores were at least 15 points (1 *SD*) below NVI, which aligns with results reported by Mei et al. (2018), indicating that average language scores were almost 1 *SD* below NVI. Additionally, the lowest possible CLS was obtained by 30% (7/23) of children with 16p11.2DS and 50% (5/10) of children with 16p11.2Dup. This suggests that the true difference between language and NVI might be even larger and that language is more impaired than what would be expected based on their cognitive level. Despite these language impairments, their nonverbal reasoning and problem-solving skills, such as the ability to understand and interpret visual information and recognize patterns, seem to be less impaired. The presence of a considerable subgroup achieving the lowest scores could also indicate potential floor effects in the current language tests. When some children obtain very low raw scores, it becomes challenging to ascertain whether their scores accurately reflect their comprehension of the subtest instructions or if their language skills were insufficient to complete the task. Therefore, it is important to interpret language skills in the context of their broader cognitive profile.

Influence of Confounding Factors on Language Outcomes

Our analysis revealed a significant influence of nonverbal cognition on language outcomes in 16p11.2Dup, with almost all variability in language outcomes (92.3%) explained by variations in nonverbal cognitive skills. Surprisingly, this influence was not significant in the deletion group. Similarly, autistic traits did not exert a significant impact on language outcomes in either CNV group. This suggests that although autistic traits are characteristic of individuals with 16p11.2 CNVs, they may not be the primary determinants of language abilities. These findings are partially consistent with those of Kim et al. (2020),

who found that cognitive skills were significant predictors of language outcomes in both CNV groups, with minimal to no influence from ASD diagnosis. In children with 16p11.2DS, language deficits persisted even after adjusting for autistic traits and nonverbal intelligence, aligning with the findings of Kim et al. In individuals with 16p11.2Dup, language skills were still significantly poorer compared to the normative sample, although the difference was smaller (within 1 *SD* of the mean). This partially aligns with the results of Kim et al., who primarily reported persistent language difficulties in certain pragmatic language skills among children with 16p11.2Dup.

Overall, these findings highlight the complexity of the relationship between genetic factors, cognitive abilities, and language development. Further research is needed to elucidate the underlying mechanisms and pathways through which these factors interact to shape language outcomes in individuals with 16p11.2 CNVs. Such insights could have important implications for the development of targeted interventions and support strategies tailored to the specific needs of individuals with these genetic variations.

Strengths, Limitations, and Future Directions

The current study has several strengths, including the focus on two distinct CNV groups, 16p11.2DS and 16p11.2Dup, which facilitates cross-CNV comparisons to discern syndrome-specific characteristics. The inclusion of unaffected siblings as a control group for 16p11.2DS minimized the influence of contextual variables including SES and parental educational levels. Additionally, it provided valuable insights into the interplay of environmental and genetic components that could have an influence on cognitive, language, and behavioral skills in 16p11.2DS. The use of standardized language and intelligence tests further strengthens the study by allowing for comparisons with the normative sample. A final key strength is that we controlled for two relevant confounding factors: social responsiveness skills and nonverbal intelligence.

However, the relatively small and clinically ascertained cohort limits our capacity to draw broad conclusions about the entire 16p11.2 CNV population. Despite this limitation, we still observed significant differences between children with 16p11.2DS and their unaffected siblings, highlighting the robustness of the results. The absence of genome or trio whole exome sequencing for children with 16p11.2 CNVs raises the possibility of additional (likely) pathogenic variants. In addition, the restricted inclusion of index patients may introduce bias into the findings. To address these limitations, future studies would benefit from larger, multisite studies and including carrier relatives identified through segregation analysis. Despite these constraints, the current study significantly contributes to our understanding of language skills in school-age children with 16p11.2 CNVs.

An extension of the current study could involve comparing profiles of children with 16p11.2Dup to those of their unaffected siblings to consider the influence of the broader familial and genetic background. Given that this study focused on lexico-semantic and morphosyntactic language abilities, future research should also characterize pragmatic language skills and speech in both CNV groups. Furthermore, longitudinal studies are warranted to capture language skills over time, as language and cognitive profiles in CNVs may evolve over time (Swillen & McDonald-McGinn, 2015; Verbesselt, Zink, et al., 2022). While the current study explored the influence of comorbid NDDs, sex, and inheritance pattern, future studies in larger cohorts should incorporate these confounding factors through linear models. As suggested by Mei et al. (2018), future studies could also explore the potential association between more severe phenotypic features in a subset of children with 16p11.2, such as minimal verbal output, and the "two-hit" model proposed by Girirajan et al. (2010). This model suggests that severe phenotypic features arise from a second hit, such as environmental influences, gene mutations, or a second CNV. Although the model has been used in the context of children with 16p11.2DS (Brisset et al., 2015), its applicability in explaining language skills remains to be established.

Conclusions

The current study characterized language profiles of school-age children with 16p11.2 CNVs, in relation to the normative sample and unaffected siblings of children with 16p11.2DS. Severe language deficits were found in the majority of children with 16p11.2 CNVs, suggesting a language profile where multiple language domains are impaired, rather than a syndrome-specific pattern targeting specific subdomains. Language deficits persisted predominantly in children with 16p11.2DS, even after controlling for autistic traits and nonverbal intelligence, whereas language profiles in 16p11.2Dup were mainly influenced by nonverbal intelligence.

From a clinical point of view, it is recommended to regularly monitor language development in children with 16p11.2 CNVs. As suggested by Chung et al. (2024), early screening and assessment of language abilities in both CNVs are advised to provide educational assistance in school and/or speech-language therapy through rehabilitation centers or private practitioners. Because there is variability present in language performances, tailored and individualized interventions are needed to enhance language abilities and mitigate potential long-term impacts of language and communication difficulties.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Standards

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee Research of University Hospitals Leuven (protocol code: S54485; December 6, 2012, and March 26, 2021). Patients and their parents were directly informed about the aims of the research project, and all participants signed informed consent.

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