Deep phenotyping of language, cognition and behaviour in children with recurrent copy number variants

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Deep phenotyping of language, cognition and behaviour in school-aged children with recurrent copy number variants

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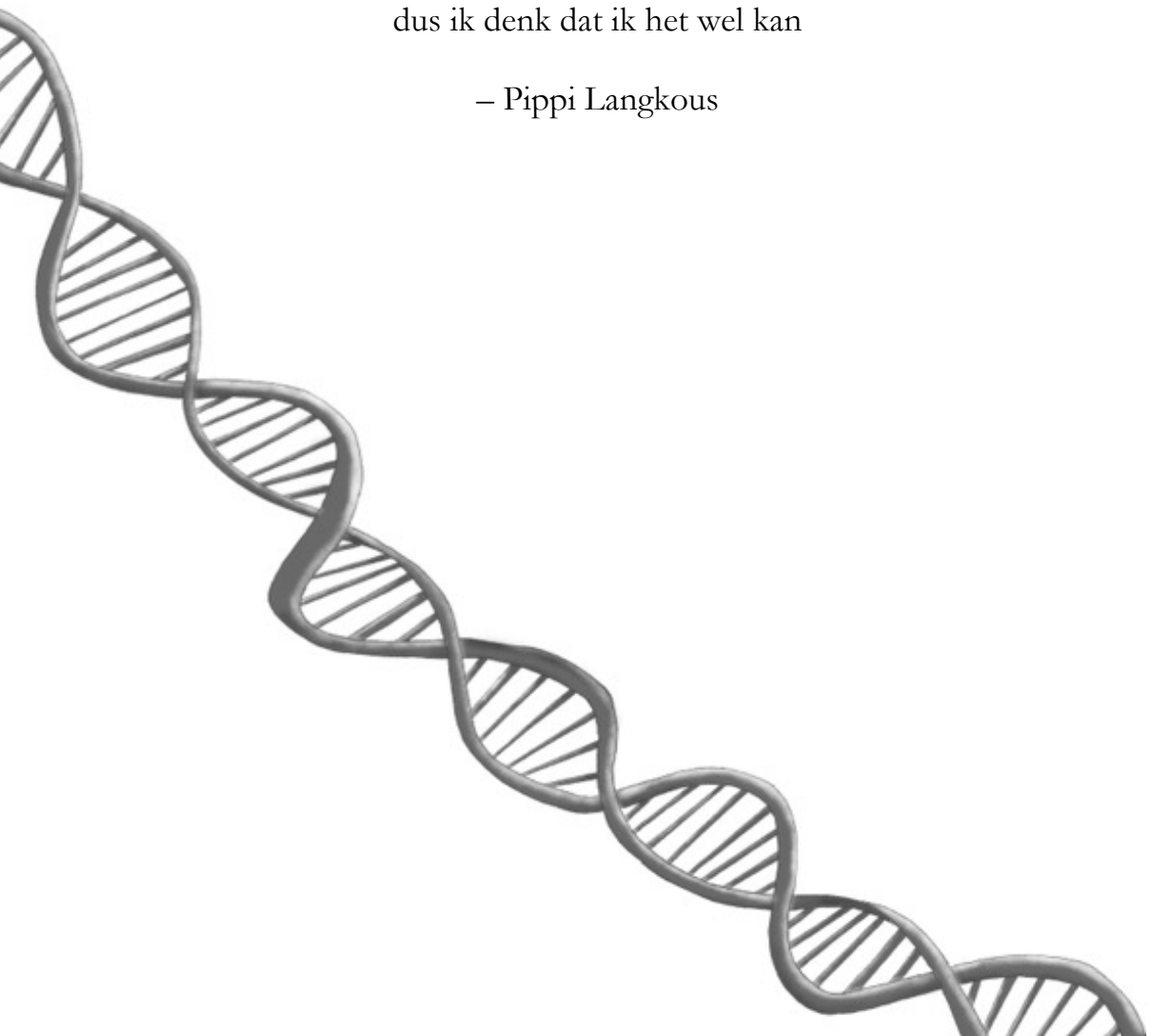
Ik heb het nog nooit gedaan,
dus ik denk dat ik het wel kan

– Pippi Langkous



Summary

Recurrent copy number variants (CNVs), which are structural genetic variations involving deletions or duplications, confer risk for neurodevelopmental disorders (NDDs). Neurodevelopmental disorders refer to a broad spectrum of impairments in cognitive, communicative, language, social, behavioural, and motor development, occurring in the first decades of life. The current dissertation focused on four recurrent CNVs: 22q11.2 deletion (22q11.2DS), 22q11.2 duplication (22q11.2Dup), 16p11.2 deletion (16p11.2DS) and 16p11.2 duplication (16p11.2Dup). These are among the most common structural variants that confer significant risk for NDDs across the lifespan (NDD-CNVs). The in-depth characterisation of language, cognition and behaviour is crucial for understanding the nature, occurrence and severity of neurodevelopmental difficulties associated with these four CNVs. This understanding is needed to inform healthcare professionals, and guide neurodevelopmental follow-up and intervention strategies aimed at mitigating the potential long-term impact of these difficulties. However, structured, protocol-driven studies into language, behavioural and cognitive difficulties have remained fragmented, lacking clear differentiation between language and speech deficits, particularly in 22q11.2Dup and 16p11.2 CNVs. Furthermore, there has been a lack of research exploring the relationship between speech-language difficulties and concurrent developmental and behavioural problems within these NDD-CNVs.

The main aim of the current dissertation was to delineate and characterise language, social, behavioural and cognitive profiles in a clinically ascertained cohort of school-aged children with 22q11.2DS, 22q11.2Dup, 16p11.2DS and 16p11.2Dup, as well as investigate potential associations between these neurodevelopmental areas, through deep phenotyping. Deep phenotyping involves the comprehensive examination of phenotypic features, including the detailed observation and description of individual components of the phenotype. Six studies were performed in an attempt to unravel the neurodevelopmental and behavioural phenotypes of these four recurrent NDD-CNVs.

In the first study (Chapter 2), we characterised clinical, behavioural and cognitive features in individuals with 22q11.2Dup. Frequent clinical symptoms encompassed nutritional issues, failure to thrive, transient hearing impairment,

and congenital heart defects. Speech-language, developmental and motor delays were prevalent during infancy, while attention, learning, motor and language difficulties were mostly reported during primary school years. Median full-scale IQ fell within the borderline range, with 21% exhibiting mild intellectual disability. Longitudinal analysis of IQ scores revealed that one-third showed a growing into deficit trajectory, indicated by an IQ score at the second time point that was at least 10 points lower than the score at the first time point.

In the second study (Chapter 3), we aimed to identify social-communicative challenges in children with 22q11.2Dup compared to their unaffected siblings and age-matched children with 22q11.2DS. Parents reported that both 22q11.2 CNV groups showed more social-communicative difficulties compared to the normative sample, whereas children with 22q11.2Dup seemed to occupy an intermediary position between their siblings and children with 22q11.2DS. In comparison to 22q11.2DS, children with 22q11.2Dup were reported to demonstrate less frequent and less severe difficulties. In addition, parents reported more variable social-communicative outcomes, with significantly reduced repetitive behaviours and restricted interests.

In the third study (Chapter 4), we characterised language profiles in children with 22q11.2Dup, compared to age-matched children with 22q11.2DS. Mean language skills were higher in children with the 22q11.2Dup in comparison to those with 22q11.2DS, though the difference did not reach statistical significance. Children with 22q11.2 CNVs experienced significantly more language problems in relation to the general population. Children with 22q11.2DS experienced language deficits starting at the word level, while the most encountered language difficulties of children with 22q11.2Dup started at the sentence level. Both receptive and expressive language in morphosyntactic and lexico-semantic areas were affected in 22q11.2 CNV populations.

In the fourth study (Chapter 5), we aimed to identify the prevalence, nature and severity of, and the association between social-communicative and behavioural challenges in children with 16p11.2 CNVs. Compared to the general population, children with 16p11.2DS showed a high prevalence of social responsiveness and communication problems, while approximately half displayed behavioural problems. Children with 16p11.2Dup demonstrated even higher rates of social-communicative problems with significantly more externalising and overall behavioural challenges. In both CNV groups, there was

a strong positive association between behavioural and social-communicative skills.

In the fifth study (Chapter 6), we characterised developmental milestones, cognitive profiles and longitudinal cognitive trajectories in children with 16p11.2DS. Motor, language, and continence milestones were delayed. Average IQ fell within the borderline range. Both intra- and interindividual variability were found across the five cognitive domains with significant discrepancies between verbal and non-verbal skills in half. Longitudinal IQ-data indicated that school-aged children with 16p11.2DS performed statistically significantly poorer at the most recent time point with 58% demonstrating a growing into deficit profile.

In the sixth study (Chapter 7), we aimed to delineate language abilities of school-aged children with 16p11.2DS CNVs, compared to the normative sample and unaffected siblings of children with 16p11.2DS. Both 16p11.2 CNVs exhibited significantly poorer language skills compared to the normative sample and unaffected siblings of children with 16p11.2DS. No significant differences were found between children with 16p11.2DS and those with 16p11.2Dup. Severe language impairments 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 only in children with 16p11.2DS. Non-verbal intelligence only had an influence on language outcomes in children with 16p11.2Dup.

When children are diagnosed with these NDD-CNVs prenatally or early in life, healthcare professionals should be aware of the high risk of language, social, behavioural and cognitive problems. The current findings underscore the importance of early neurodevelopmental monitoring and multidisciplinary therapy. Achieving a balance between follow-up and support is crucial to adapt to the changing and increasing needs throughout life and to adjust environmental demands accordingly. Future research on 22q11.2 and 16p11.2 CNVs should focus on phenotypic characterisations across the lifespan, with prospective and longitudinal data collection from early infancy through adulthood.

Samenvatting

Recurrente kopijvarianten of copy number variants (CNVs) zijn structurele genetische varianten, waaronder deleties of duplicaties, met een verhoogd risico op neurobiologische ontwikkelingsstoornissen (NDDs). Neurobiologische ontwikkelingsstoornissen verwijzen naar een breed spectrum van beperkingen in cognitieve, communicatieve, taal-, sociale, gedrags- en motorische ontwikkeling, die zich voordoen in de eerste decennia van het leven. Dit proefschrift richtte zich op vier recurrente CNVs: 22q11.2 deletie (22q11.2DS), 22q11.2 duplicatie (22q11.2Dup), 16p11.2 deletie (16p11.2DS) en 16p11.2 duplicatie (16p11.2Dup). Dit zijn enkele van de meest voorkomende structurele varianten die aanzienlijke risico's met zich meebrengen voor NDDs doorheen het leven (NDD-CNVs). Een grondige karakterisering van taal, cognitie en gedrag is van groot belang om de aard, het voorkomen en de ernstgraad van neurobiologische ontwikkelingsproblemen die geassocieerd zijn met deze vier CNVs beter te begrijpen. Deze inzichten zijn noodzakelijk om zorgprofessionals te informeren, en follow-up en interventiestrategieën te ontwikkelen die gericht zijn op het verminderen van de potentiële lange termijn impact van deze moeilijkheden. Gestructureerde, protocol gedreven studies naar taal-, gedrags- en cognitieve moeilijkheden zijn echter beperkt, waarbij er een gebrek is aan duidelijk onderscheid tussen taalontwikkelings- (TOS) en spraakklankstoornissen (SKS), met name bij 22q11.2Dup en 16p11.2 CNVs. Bovendien is er een gebrek aan onderzoek naar de relatie tussen spraak-taal problemen en ontwikkelings- en gedragsproblemen binnen deze NDD-CNVs.

De voornaamste doelstelling van het huidige proefschrift was om de taal-, sociale, gedrags- en cognitieve profielen in kaart te brengen en te karakteriseren in een klinisch cohort van schoolgaande kinderen met 22q11.2DS, 22q11.2Dup, 16p11.2DS en 16p11.2Dup, en om mogelijke verbanden tussen deze neurobiologische ontwikkelingsdomeinen te onderzoeken door gebruik te maken van diepe fenotypering. Diepe fenotypering omvat het uitgebreid onderzoek van fenotypische kenmerken, waaronder de gedetailleerde observatie en beschrijving van individuele componenten van het fenotype. Zes studies werden uitgevoerd om de neurobiologische ontwikkelings- en gedragsfenotypes van deze vier recurrente NDD-CNVs te ontrafelen.

In de eerste studie (Hoofdstuk 2) hebben we klinische, gedrags- en cognitieve kenmerken gekarakteriseerd bij personen met 22q11.2Dup.

Veelvoorkomende klinische symptomen omvatten voedingsproblemen, failure to thrive, voorbijgaande gehoorproblemen en aangeboren hartafwijkingen. Spraak-taal-, ontwikkelings- en motorische vertragingen kwamen vaak voor tijdens de kindertijd, terwijl aandachts-, leer-, motorische en taalproblemen voornamelijk tot uiting kwamen tijdens de lagere schooljaren. De mediaan van het totale IQ viel binnen het zwakbegaafde bereik, waarbij 21% een lichte verstandelijke beperking vertoonde. Longitudinale analyse van de IQ scores toonde aan dat één derde een "growing into deficit" traject vertoonde, waarbij de IQ score op het tweede testmoment minstens 10 punten lager was dan op het eerste testmoment.

In de tweede studie (Hoofdstuk 3) richtten we ons op de identificatie van sociaal-communicatieve uitdagingen bij kinderen met 22q11.2Dup vergeleken met hun broers en zussen zonder CNV en leeftijdsgenoten met 22q11.2DS. Ouders rapporteerden dat beide groepen met 22q11.2 CNV meer sociaal-communicatieve moeilijkheden vertoonden in vergelijking met de normatieve steekproef, terwijl kinderen met 22q11.2Dup een tussenpositie leken in te nemen tussen hun broers en zussen enerzijds en kinderen met 22q11.2DS anderzijds. In vergelijking met 22q11.2DS hadden ouders van kinderen met 22q11.2Dup minder vaak en minder ernstige bezorgdheden. Daarnaast rapporteerden ze meer variabele sociaal-communicatieve uitkomsten, gekenmerkt door significant minder stereotiepe gedragingen en preoccupaties in vergelijking met 22q11.2DS.

In de derde studie (Hoofdstuk 4) hebben we taalprofielen in kaart gebracht bij kinderen met 22q11.2Dup, in vergelijking met leeftijdsgenoten met 22q11.2DS. Gemiddelde taalvaardigheden waren beter bij kinderen met 22q11.2Dup in vergelijking met kinderen met 22q11.2DS, maar dit verschil was niet statistisch significant. Kinderen met 22q11.2 CNVs ervaarden significant meer taalproblemen in vergelijking met de algemene populatie. Kinderen met 22q11.2DS hadden reeds taaltekorten op woordniveau, terwijl de meest voorkomende taalproblemen bij kinderen met 22q11.2Dup voorkwamen op zinsniveau. Zowel receptieve als expressieve taal, en morfosyntactische als lexicosemantische vaardigheden waren aangetast in de 22q11.2 CNV-populaties.

De vierde studie (Hoofdstuk 5) had als doel om de prevalentie, aard en ernst van, en de associatie tussen sociaal-communicatieve en gedragsmatige uitdagingen bij kinderen met 16p11.2 CNVs te karakteriseren. In vergelijking met de algemene populatie vertoonden kinderen met 16p11.2DS een hoge prevalentie

van problemen met sociale responsiviteit en communicatie, terwijl ongeveer de helft gedragsproblemen vertoonde. Kinderen met 16p11.2Dup vertoonden zelfs meer sociaal-communicatieve problemen met significant meer externaliserende en algemene gedragsproblemen. In beide CNV-groepen was er een sterk positief verband tussen gedrags- en sociaal-communicatieve vaardigheden.

In de vijfde studie (Hoofdstuk 6) karakteriseerden we ontwikkelingsmijlpalen, cognitieve profielen en longitudinale cognitieve trajecten bij kinderen met 16p11.2DS. Motorische, taal- en zindelijkheidsmijlpalen waren vertraagd. Het gemiddelde IQ viel binnen het zwakbegaafde bereik. Zowel intra- als interindividuele variabiliteit werd gevonden in de vijf cognitieve domeinen, met significante verschillen tussen verbale en non-verbale vaardigheden bij de helft van de kinderen. Longitudinale IQ-gegevens toonden aan dat schoolgaande kinderen met 16p11.2DS statistisch significant slechter presteerden op het meest recente tijdstip, waarbij 58% een "growing into deficit" profiel vertoonde.

De doelstelling van de zesde studie (Hoofdstuk 7) was om taalvaardigheden in kaart te brengen bij schoolgaande kinderen met 16p11.2DS CNVs, in vergelijking met de normatieve steekproef en broers en zussen zonder CNV van kinderen met 16p11.2DS. Beide 16p11.2 CNVs vertoonden significant slechtere taalvaardigheden ten opzichte van de normatieve steekproef en broers en zussen zonder CNV van kinderen met 16p11.2DS. Er werden geen significante verschillen gevonden tussen kinderen met 16p11.2DS en die met 16p11.2Dup. Ernstige taalproblemen werden geïdentificeerd bij 70% van de kinderen met 16p11.2 CNVs in alle taaldomeinen, waarbij beide groepen significant betere receptieve woordenschatvaardigheden vertoonden dan algemene receptieve taalvaardigheden. Expressieve taalproblemen waren alleen bij kinderen met 16p11.2DS meer uitgesproken dan receptieve taalproblemen, terwijl non-verbale intelligentie alleen invloed had op taalresultaten bij kinderen met 16p11.2Dup.

Wanneer kinderen prenataal of op jonge leeftijd gediagnosticeerd worden met deze NDD-CNVs, is het belangrijk dat zorgprofessionals zich bewust zijn van het hoge risico op taal-, sociale, gedrags- en cognitieve problemen. De huidige bevindingen benadrukken het belang van vroegtijdige opsporing en follow-up van neurobiologische ontwikkelingsproblemen en multidisciplinaire therapie. Het is van belang om een evenwicht te vinden tussen opvolging en ondersteuning om te kunnen inspelen op de veranderende en groeiende behoeften gedurende verschillende levensfasen, en om de

verwachtingen van de omgeving bij te stellen. Toekomstig onderzoek naar 22q11.2 en 16p11.2 CNVs moet zich richten op de fenotypische karakterisering gedurende het leven, met prospectieve en longitudinale gegevensverzameling vanaf de vroege kindertijd tot volwassenheid.

Abbreviations

16p11.2DS	16p11.2 deletion syndrome
16p11.2Dup	16p11.2 duplication
22q11.2DS	22q11.2 deletion syndrome
22q11.2Dup	22q11.2 duplication
AAC	Alternative and augmentative communication
ABA	Applied behaviour analysis
AD	Anxiety disorder
ADD	Attention deficit disorder
ADHD	Attention-deficit/hyperactivity disorder
ADI-R	Autism Diagnostic Interview – Revised
ADOS-2	Autism Diagnostic Observation Schedule – Second Edition – Dutch version
Array CGH	Array comparative genomic hybridisation
ASD	Autism spectrum disorder
AVL	ADHD vragenlijst Dutch version
BAC	Bacterial Artificial Chromosome
BMI	Body mass index
BOT-2	Bruininks-Oseretsky Test of Motor Proficiency - Second Edition
BP	Breakpoints
BPD	Bipolar disorder
BSID-II-NL	Bayley Scales of Infant Development – Second edition
CA	Chronological Age
CAS	Childhood apraxia of speech
CBCL	Child Behavior Checklist
CCC-2-NL	Children's Communication Checklist – Second Edition – Dutch version
CELF-3	Clinical Evaluation of Language Fundamentals Third edition
CELF-4-NL	Clinical Evaluation of Language Fundamentals Fourth edition - Dutch version
CELF-5	Clinical Evaluation of Language Fundamentals Fifth edition
CELF-P2-NL	Clinical Evaluation of Language Fundamentals - Preschool – Second Edition. Dutch version
CFD	Concepts and Following Directions
CHC	Cattell-Horn-Carroll
CHD	Congenital heart disease/defects
CHOP	Children's Hospital of Philadelphia
CLS	Core Language Score

CMA	Chromosomal microarray
CME/CHG	Centrum Menselijke Erfelijkheid – Centre for Human Genetics
CNV	Copy Number Variant
CP	Cerebral palsy
CVI	Cerebral visual impairment
CVIT	Children's Visual Impairment Test Dutch version
DAS-II	Differential Ability Scales – 2nd Edition
dB HL	Decibel Hearing Level
DCD	Developmental coordination disorder
DLD	Developmental language disorder
DQ	Developmental quotient
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, fifth version
ECHO	Experiences of Children with Copy Number Variants
EC Research	Ethics Committee Research
ELI	Expressive language index
ENT	Ear nose throat
EV	Expressive Vocabulary
FDR	False Discovery Rates
FECMP	Families experiencing complex and multiple problems
FISH	Fluorescence In Situ Hybridisation
FRI	Fluid Reasoning Index
FS	Formulated Sentences
FSIQ	Full-scale IQ
G2MH network	Genes to mental health network
Geisinger ADMI	Geisinger Autism & Developmental Medicine Institute
GCC	General Communication Composite
GRCh38	Genome reference consortium human build 38
GS	Genome sequencing
Hg19	Humane genome 19
HL	Hearing loss
HPO	Human phenotype ontology
IBBC	International 22q11.2 brain behavior consortium
ID	Intellectual disability
IQ	Intelligence Quotient
IRB	Institutional review board
ISCED	International Standard Classification of Education
KABC-II	Kaufman Assessment Battery for Children – 2nd Edition
kb	kilobase
KBIT-2	Kaufman Brief Intelligence Test – Second Edition
LCR	Low copy repeat
LD	Language disorder

Mb	Megabases
MDD	Major depressive disorder
ML5LU	Mean Length 5 Longest Utterances
MLPA	Multiplex ligation-dependent probe amplification
MLU	Mean Length of Utterance
Movement ABC-2	Movement Assessment Battery for Children - Second Edition
MRI	Magnetic Resonance Imaging
NAHR	Non-allelic homologous recombination
N-CDI	MacArthur Communicative Development Inventories - Dutch version
NDD	Neurodevelopmental disorder
NDD-CNV	CNVs associated with NDD
NGS	Next-generation sequencing
NNST	Dutch version of the Nonspeech test
NVI	Nonverbal Index
NVIQ	Non-verbal IQ
OMIM	Online Mendelian Inheritance in Man
PC	Pragmatic Composite
PDMS-2	Peabody Developmental Motor scales – Second Edition
PIQ	Performance IQ
PJLO	Preschool Judgement of Line Orientation
PPVT-III-NL	Peabody Picture Vocabulary Test- Third Edition – Dutch version
PSI	Processing Speed Index
PTONI	Primary Test of Nonverbal Intelligence
RIB	Restricted Interests and Behaviour
RLI	Receptive language index
RTNA	Renfrew language scales Dutch version
RTOS	Reynell Developmental Language Scales Dutch version
RS	Recalling Sentences
SA	Sentence Assembly
SCI	Social Communication and Interaction
SCZ	Schizophrenia
SD	Standard deviation
SES	Socioeconomic status
Simons VIP	Simons Variation in Individuals Project
SLD	Specific learning disorder
SNP	Single nucleotide polymorphism
SON-R	Snijders – Oomen Nonverbal test Revised
SR	Semantic Relationships
SRO	Small region of overlap
SRS-NL	Social Responsiveness Scale – Dutch Version

SRS-2-NL	Social Responsiveness Scale – Second Version - Dutch
SS	Scaled scores
SSD	Speech sound disorder
SST	Sentence Structure
STTP	Schlichting Test for Language Production Dutch version
TEA-Ch	Test of Everyday Attention for Children Dutch version
TGMD-2	Test of Gross Motor Development- Second Edition
TRF	Teacher's Report Form
TVPS-R	Test of visual-perceptual skills (non-motor) – Revised Dutch version
UNESCO	United Nations Educational, Scientific and Cultural Organisation
UZ Leuven	University Hospitals Leuven (Universitair Ziekenhuis)
VCFS	Velocardiofacial syndrome
VCI	Verbal Comprehension Index
VIQ	Verbal IQ
VISK	Vragenlijst voor Inventarisatie van Sociaal gedrag voor Kinderen Dutch version
VMI-Beery	The Beery-Buktenica Developmental Test of Visual-Motor Integration
VPI	Velopharyngeal insufficiency
VSI	Visual Spatial Index
VUS	Variant of uncertain significance
WASI-II	Wechsler Abbreviated Scale of Intelligence – Second Edition
WC	Word Classes
WD	Word Definitions
WCS	Word comprehension score
WES	Whole exome sequencing
WHO	World Health Organisation
WISC-III-NL	Wechsler Intelligence Scale for Children – Third Edition
WISC-V-NL	Wechsler Intelligence Scale for Children – Fifth Edition
WMI	Working Memory Index
WNV-NL	Wechsler Nonverbal Scale of Ability
WPPSI-III-NL	Wechsler Preschool and Primary Scale of Intelligence - Third Edition Dutch version
WPPSI-IV-NL	Wechsler Preschool and Primary Scale of Intelligence – Fourth Edition
WPPSI-R-NL	Wechsler Preschool and Primary Scale of Intelligence Revised Dutch version
WS	Word Structure

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Chapter 1

Chapter 1 - General introduction

1.1 Rationale for the current dissertation

Recurrent pathogenic copy number variants (CNVs), which are structural genetic variations involving deletions or duplications, are associated with elevated risk for neurodevelopmental disorders (NDDs) (Zarrei et al., 2019). So far, about 70 recurrent CNVs have been broadly linked to NDDs, collectively accounting for approximately 15% of individuals diagnosed with NDDs (G. M. Cooper et al., 2011; Forrest & Penzes, 2023; Mollon et al., 2023). Neurodevelopmental disorders constitute a range of conditions marked by impairments in cognitive, language and communicative, behavioural, and motor development and can occur starting from conception to early adulthood (Grayton et al., 2012; Lee & Lupski, 2006; A. Moreno-De-Luca et al., 2013). Recurrent CNVs at chromosomal loci 22q11.2 and 16p11.2, including 22q11.2 deletion/duplication (22q11.2DS – 22q11.2Dup) and 16p11.2 deletion/duplication (16p11.2DS – 16p11.2Dup), are among the most common structural variants that confer significant risk for NDDs across the lifespan (NDD-CNVs). In addition, these NDD-CNVs are among the most frequently observed variants in patient samples with NDD and in the general population (Deshpande & Weiss, 2018; Goldenberg, 2018; Lowther et al., 2017).

The focus of the current dissertation is to delineate and characterise language, behavioural and cognitive profiles in school-aged children with 22q11.2DS, 22q11.2Dup, 16p11.2DS and 16p11.2Dup and to investigate whether an association exists between these neurodevelopmental areas in the selected NDD-CNVs, through deep phenotyping. Deep phenotyping of language, cognition and behaviour is important for understanding the nature, occurrence and severity of neurodevelopmental difficulties associated with these four CNVs.

Systematic, protocol-driven research on language, behavioural and cognitive difficulties is rather fragmentary and no clear distinction has been made between speech and language impairments, particularly in 22q11.2Dup and 16p11.2 CNVs. Language and speech are relevant research topics because of their link to and comorbidity with cognition, behaviour, socio-emotional development, everyday communication, academic performance and quality of life

(Van Agt et al., 2011; Vyshedskiy et al., 2017). In addition, almost no research exists on the association between speech-language problems and comorbid developmental and behavioural disorders in these NDD-CNVs. Therefore, studies with standardised language, developmental and behavioural assessments are required to get more insight in the phenotype of these four recurrent CNV populations, and into the interactions between these neurodevelopmental areas. In the next paragraphs, the most important concepts for this dissertation will be introduced and discussed: Neurodevelopment and neurodevelopmental disorders (1.2), Deep phenotyping (1.3), and Copy number variants (1.4). Then, the four selected CNVs with elevated risk for NDDs will be described (1.5). The final part of this chapter (1.6) focusses on the research objectives, participants, protocol and the outline of the conducted studies.

1.2 Neurodevelopmental outcome and neurodevelopmental disorders (NDDs)

According to the lifespan perspective, neurodevelopment is a lifelong, multidimensional and multidirectional process, characterised by high plasticity and influenced by various interacting factors (Berk, 2018; Karmiloff-Smith, 2012a). Neurodevelopment is a perpetual journey, meaning that every period, from prenatal to late adulthood (>65 years) can significantly impact changes across different interconnected, overlapping and interacting neurodevelopmental domains. Each major period comes with its own challenges and opportunities, resulting in shared neurodevelopmental characteristics for everyone. Notwithstanding, the demands individuals experience and the changes they undergo vary in terms of timing and pattern (Berk, 2018). To gain a comprehensive understanding of neurodevelopment in both typically and atypically developing individuals, it is essential to track neurodevelopmental trajectories over time, given the dynamic and evolving nature of neurodevelopment across the entire lifespan (Cornish et al., 2007; Karmiloff-Smith, 2012a). Multidimensionality refers to a combination of different neurodevelopmental domains (see 1.2.2), whereas multidirectionality implies that neurodevelopment is not always about growth or making progress; both improved and reduced functioning might occur over time and in each of these neurodevelopmental domains. In addition, both continuous and discontinuous developmental changes can transpire. Although plasticity gradually decreases over time, neurodevelopment remains plastic at every age, providing the opportunity to navigate diverse life events (Berk, 2018).

In summary, neurodevelopmental outcome and trajectories are shaped by a complex interplay of multiple factors, including individual-specific risk and protective factors, familial and environmental influences, and the progression of time and development itself (see Figure 1.1). These factors operate independently, accumulate over time, and synergistically contribute to the overall outcome (Brown et al., 2020; Lein, 2015; Swillen et al., 2018). Risk factors refer to those that have been consistently associated with negative neurodevelopmental outcomes, whereas protective factors help individuals to reduce impact of risk factors or adverse environments (de Voursney et al., 2008). All factors relate to either nature or nurture or result from complex interactions between both. Nature refers to the hereditary information individuals inherit from their parents, which influences our biological traits, characteristics and predispositions. Nurture encompasses environmental factors from both the physical and social worlds, shaping our biological composition and psychological experiences pre- and postnatally (Berk, 2018). Increasing evidence from an interactionist approach suggests that the connection between nature and nurture operates as a bidirectional pathway: genes influence individual's behaviour and experiences, while experiences and behaviour reciprocally impact gene expression. This phenomenon is called epigenesis, referring to development arising from continuous, bidirectional interactions between heredity and all facets of the environment (Berk, 2018). Illustrated by an example from the language domain, several research groups (Kraft & DeThorne, 2014; Rice, 2012; S. D. Smith, 2011) have proposed epigenetic regulatory mechanisms as likely contributors to Developmental Language Disorders (DLDs) (Mountford et al., 2022). This suggests a plausible layer of genetic control that could contribute to the complexity and heterogeneity of language disorders. Despite this, no studies have yet established a clear link between epigenetic regulation and DLDs (Mountford et al., 2022).

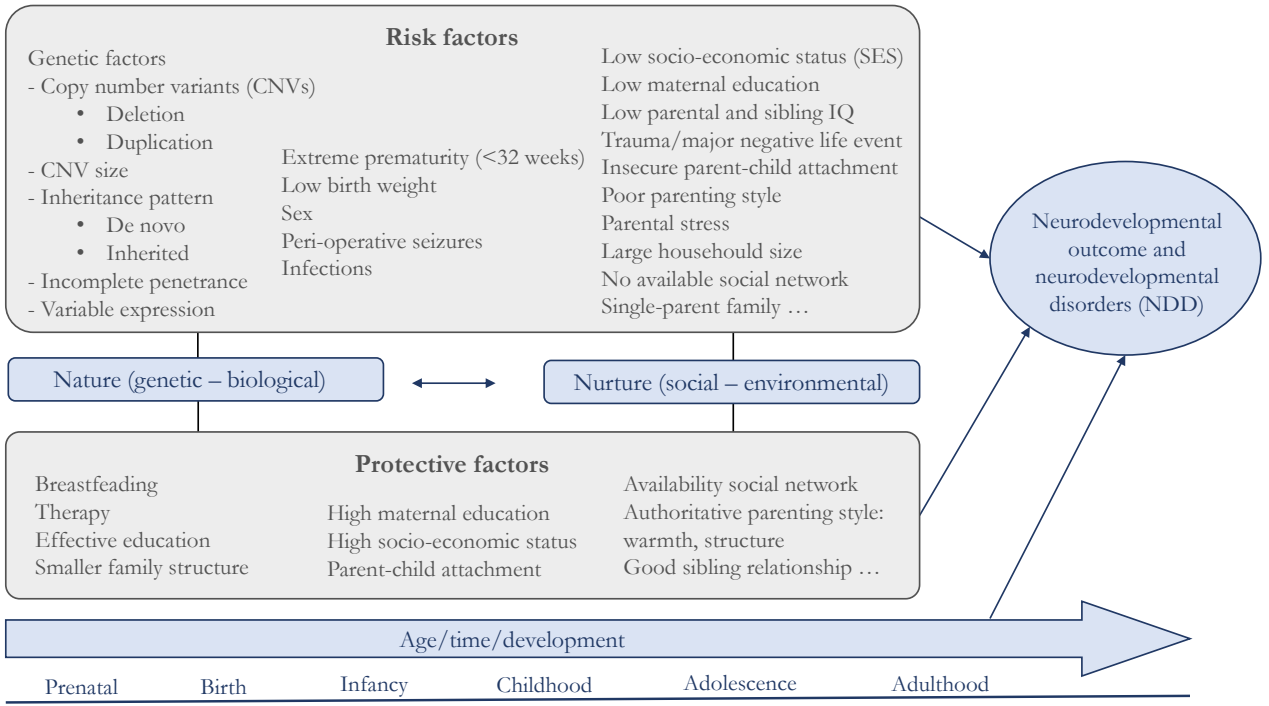


Figure 1.1 – Potential factors playing a role in neurodevelopment and neurodevelopmental disorders.
The contribution of various individual and familial/environmental risk and protective factors, and time/development result in a certain neurodevelopmental outcome. Figure adapted from Swillen et al., 2018, and de Voursney et al., 2008.

1.2.1 Neurodevelopmental disorders

Neurodevelopmental disorders (NDD) refer to a set of conditions with onset in the developmental period, leading to deficiencies in the central nervous system and causing impairments in functioning across neurodevelopmental domains (Morris-Rosendahl & Crocq, 2020). Epidemiological findings indicate that NDDs more often co-occur, and thus have a higher comorbidity, than would be expected by chance. These studies also suggest that NDDs should be interpreted as distinct patterns of impairments or symptoms of a common underlying neurodevelopmental spectrum (Kiser et al., 2015). Moreno-De-Luca et al. (2013) introduced a conceptual framework with the developmental brain dysfunction as the shared factor that underlies a wide continuum of neurodevelopmental difficulties and disorders, as displayed in Figure 1.2. Developmental brain dysfunction can be caused by genetic factors such as a CNV or by an insult to the developing central nervous system, like teratogen exposures, infections, nutritional deficiencies or traumatic events. Deviant or disrupted brain development results in cognitive, behavioural, motor and communicative difficulties, with the specific pattern of issues determining the clinical diagnosis of NDD. This model proposes that each causal factor can lead to a continuum of impairments with variable severity (A. Moreno-De-Luca et al., 2013; Morris-Rosendahl & Crocq, 2020; Myers, 2013).

In international classification systems such as the Diagnostic and Statistical Manual of Mental Disorders, fifth version (DSM-5), NDDs include seven subdivisions of focus: intellectual disability (ID); communication disorders (CD), including developmental language disorders (DLD) and speech sound disorders (SSD); autism spectrum disorder (ASD); attention-deficit/hyperactivity disorder (ADHD); neurodevelopmental motor disorders, including tic disorders and developmental coordination disorder (DCD); and specific learning disorders (SLD) (American Psychiatric Association, 2013). In literature, NDDs also extend to comprise neuropsychiatric disorders that emerge in adolescence or adulthood, such as bipolar disorder (BPD), mood disorders, anxiety disorders and schizophrenia (Kim & State, 2014). Additionally, NDDs also encompass conditions that fall outside the DSM-5 classification, such as cerebral palsy (CP) and epilepsy (Johnson & Shorvon, 2011; A. Moreno-De-Luca et al., 2013; Rapoport et al., 2012; A. L. Reiss, 2009). This broad definition of NDDs will be used throughout this dissertation (van der Werf et al., 2020). NDDs affect >3% of the population and typically have a lasting impact on individuals across their lifespan (Bosch et al., 2022; Mitani et al., 2021; Swillen, 2024). These disorders

exhibit a complex and dynamic nature, showing variations in degree and severity. Moreover, the course of the clinical presentation and symptoms differs depending on the chronological age and developmental stage of the child (Brown et al., 2020; Swillen, 2024).

It is important to acknowledge that the DSM-5 primarily adopts a medical model view of impairment. However, alternative perspectives, such as the social model of disability and the neurodiversity model, offer valuable insights. In the medical model, disability is perceived as resulting from various levels of impairment, implying that disabilities are considered inherently negative conditions that require curing or at least treatment. Conversely, the social model proposes that societal barriers are the primary cause of disability. Specifically, disabilities arise from society's failure to accommodate an individual's impairments, rather than being inherent impairments in the individual (Adam & Koutsoklenis, 2023; Nicholls, 2018; Pellicano & den Houting, 2022). The neurodiversity movement builds on the social model by suggesting that individuals may not perceive their traits as impairments. Advocates within the neurodiversity movement argue that they are different, not deficient, and that it is the responsibility of "neurotypicals" to accept these differences and manage any discomfort they may feel. Consequently, individuals within the neurodiversity model assert that they do not require treatment or cure, and any interventions should be the individual's choice rather than imposed by others (Nicholls, 2018; Oliver, 1996; Pellicano & den Houting, 2022; Vanheule, 2017). For instance, the neurodiversity paradigm diverges from the traditional medical model's view of ASD or autism as a disorder. Instead, it regards common traits in autism as neurological differences rather than deficits. This perspective shifts the focus away from a disease model and emphasises the unique strengths of autistic individuals, embracing autism as a natural variation of neurological diversity that does not require a cure (Genovese & Butler, 2023).

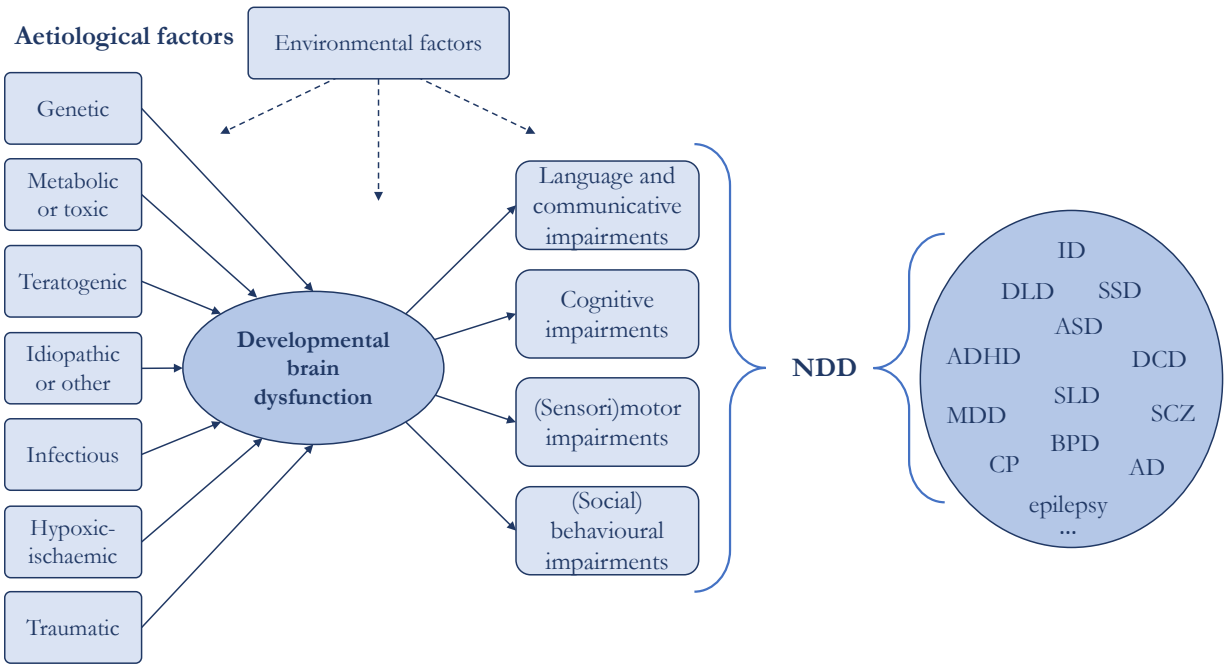


Figure 1.2 – Conceptual framework for developmental brain dysfunction.
Figure adapted from Moreno-De-Luca, 2013, and Myers, 2013. Abbreviations: ID, intellectual disability; DLD, developmental language disorder; SSD, speech sound disorder; ASD, autism spectrum disorder; ADHD, attention-deficit/hyperactivity disorder; DCD, developmental coordination disorder; SLD, specific learning disorder; MDD, major depressive disorder; SCZ, schizophrenia; BPD, bipolar disorder; CP; cerebral palsy; AD, anxiety disorder.

1.2.2 Neurodevelopmental and behavioural phenotypes

In general, phenotypes may refer to three distinct aspects: dysmorphology (e.g. facial features), medical features (e.g. cardiac disease) and neurodevelopmental and behavioural features (e.g. cognitive impairment) (O'Brien & Yule, 1995). The primary focus of the current dissertation is on the latter aspect. The term "behavioural phenotype" has been used for the past three decades in the areas of child psychiatry, developmental medicine and clinical genetics to characterise the neurobehavioural features of a condition. However, there is lack of consensus on its precise definition, and individuals may use and interpret it in various ways (Baty et al., 2011). According to Flint and Yule (1994), behavioural phenotypes are defined as a unique combination or profile of behavioural, social, language, and cognitive symptoms consistently associated with a genetic disorder (Flint & Yule, 1994; O'Brien, 2006; O'Brien & Yule, 1995). The definition implicates that the association between genotype and phenotype might be complex and variable (O'Brien & Yule, 1995). However, as hardly any feature is always associated with a certain genetic disorder, many researchers found this definition too restrictive (Skuse, 2000; Van Den Heuvel, 2016a). Therefore, the following adaption has been suggested: The general profile is observed in the majority of individuals with a given genetic disorder or syndrome (Baty et al., 2011). This aligns with the interpretation by Dykens, referring to the increased probability that individuals with a genetic condition will exhibit some behavioural or neurodevelopmental features in relation to others without this condition (Dykens & Hodapp, 2007; Waite et al., 2014).

For the current dissertation, we prefer to refer to the "neurodevelopmental and behavioural phenotype", as this aligns better with the current DSM-5 terminology. The neurodevelopmental and behavioural phenotype may involve both psychiatric and other developmental domains (Flint & Yule, 1994; O'Brien & Yule, 1995). The connection between these core domains is depicted in figure 1.3. Some neurodevelopmental and behavioural phenotypes are mainly characterised by language and cognitive difficulties, whereas others present with dominant motor and social issues. In a smaller subset of phenotypes, recognisable and diagnosable NDDs are observed (O'Brien & Yule, 1995).

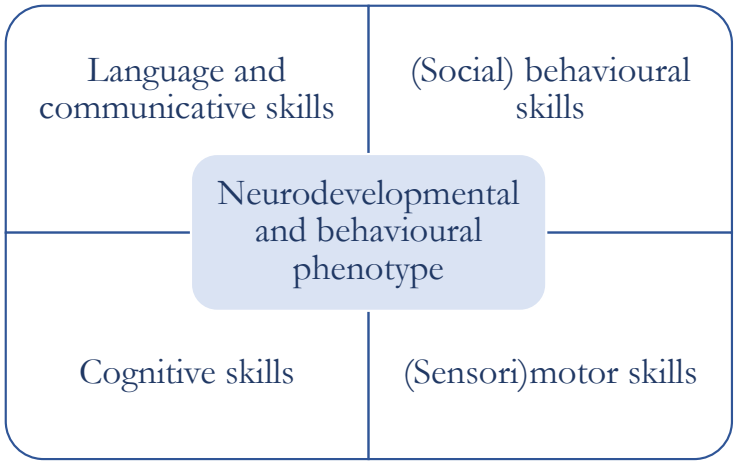


Figure 1.3 – Connection between the four core domains of the neurodevelopmental and behavioural phenotype.
Language and communicative skills, (social) behaviour skills, cognitive skills, and (sensori)motor skills. Figure adapted from O'Brien and Yule, 1995.

The "language and communicative" domain of the neurodevelopmental and behavioural phenotype is an important focus of this dissertation. Communication encompasses the exchange of information, messages, ideas, or emotions, including non-verbal cues such as gestures or vocalisations (Reed, 2018). Language is the ability to understand (comprehension/receptive skills) and use (production/expressive skills) spoken and written words and sentences (Reed, 2018; S. Reilly et al., 2015). Language comprises three primary domains: language form, language content and language use. Language form includes morphology, which refers to the rules for constructing meaningful words (e.g. plural); and syntax, which dictates the construction of meaningful phrases or sentences. Language content includes lexicon, referring to vocabulary; and semantics, which deals with the meaning and relationship among words and abstract concepts (e.g. idioms). Language use, known as pragmatics, involves adhering to rules and conventions for socially and culturally appropriate language use in context (e.g. eye contact, turn-taking) (Brown et al., 2020; Patel et al., 2011; Paul et al., 2018; Reed, 2018). Speech, distinct from language, is the oral expression of language. This process involves complex sensorimotor mechanisms orchestrated by the central nervous system, including respiration, phonation, resonation, and articulation. These physiological processes collectively facilitate the creation and modulation of sound waves, enabling the conveyance of linguistic information (Reed, 2018).

Cognition or "cognitive skills" refer to the ability of the brain to acquire, process, store, and retrieve information (Khera & Rangasamy, 2021), including memory, thinking, perception, imagination, creativity, motivation, attention, emotion recognition, executive functioning, problem solving and academic and everyday knowledge (Trivedi, 2006). Within this dissertation, the focus lies on intelligence, referring to the ability for academic and experiential learning, reasoning, planning, abstract thinking, judgement and problem-solving. Intelligence is inferred from various cognitive assessments, such as widely used intelligence tests, yielding an IQ score (Plomin & von Stumm, 2018). The Cattell-Horn-Carroll (CHC) theory of cognitive skills (intelligence) stands as a leading psychometric framework for understanding the structure of cognitive capabilities or human intelligence, integrating the contributions of Raymond Cattell, John Horn, and John Carroll. Supported by ample evidence, it serves as a foundational framework for the selection, organisation, and interpretation of intelligence and cognitive ability tests (Flanagan & Dixon, 2013). The CHC model operates on a hierarchical structure, categorising cognitive abilities into three levels: general, broad, and narrow. Initially, the g-factor (general) is subdivided into ten broad cognitive abilities (broad), including fluid reasoning (Gf), crystallised knowledge (Gc), visual processing (Gv), short-term memory (Gsm), long-term memory and retrieval (Glr), processing speed (Gs), auditory processing (Ga), quantitative ability (Gq), broad reading or writing ability (Grw), and decision or reaction speed (Gt) (Flanagan et al., 2013). These broad cognitive abilities can then be further specified into 72 specific cognitive skills (narrow), resulting in a three-tiered model (Flanagan & Dixon, 2013).

"Behavioural skills" refer to the development of the ability to regulate emotions and thoughts. Social-emotional development encompasses alterations in emotional expression, self-awareness, understanding of others, interpersonal skills, moral reasoning, formation of friendships and intimate relationships, and behaviour. It involves individuals' capacity to comprehend and manage their own emotions, effectively communicate them, and engage in behaviours that benefit others. Children's behaviours and disorders can broadly be classified based on their responses to stressors. Internalising behaviour refers to various inward-focused behaviours such as anxiety, fear, sadness/depression, social withdrawal, and somatic complaints. Conversely, externalising behaviour involves conflicts with others, aggression, conduct problems, delinquent behaviour, oppositionality, hyperactivity, and attention problems (Berk, 2018; Bosmans et al., 2015).

1.3 Deep phenotyping

The combination of alleles at a specific locus is referred to as the genotype, while the observable physical or clinical characteristics including behaviour, morphology and physiology, constitute the phenotype (Jorde et al., 2016). According to Hennekam and Biesecker (2012), the emergence of next generation sequencing tools requires the parallel development of next generation phenotyping and "deep phenotyping" to enhance our understanding of genotype-phenotype correlations (Fisch, 2018; Hennekam & Biesecker, 2012). Deep phenotyping refers to the detailed and thorough examination of phenotypic features, involving the fine-grained observation and description of individual components of the phenotype (Köhler et al., 2017; Robinson, 2012). While this definition is primarily applied in the context of the medical phenotype for precision medicine, using the accompanying human phenotype ontology (HPO), it can also be applicable to the neurodevelopmental and behavioural phenotype. For the current dissertation, deep phenotyping is defined as the comprehensive characterisation of the neurodevelopmental and behavioural features, comprising communicative, (social) behavioural, cognitive and motor skills, in specific recurrent CNV populations (see 1.6).

Many genetic disorders related with language and speech deficits have widespread impacts on the developing brain, affecting not only language but also motor and cognitive skills. However, in research, the characterisation of speech-language skills across specific genetic disorders is conducted in various ways (Chenausky & Tager-Flusberg, 2022). Descriptions of phenotypes in the literature often lack precision or specificity (Robinson, 2012; Steinman et al., 2016). For instance, "speech delay" may refer to actual speech delay, speech impairment, language delay or language impairment (Bartik et al., 2022; Ou et al., 2008; Van Campenhout et al., 2012). Language (the understanding and use of morpho-syntax, lexico-semantics and pragmatics) differs from speech (the production of speech sounds), although the two are related and have common underlying neural structures (Chenausky & Tager-Flusberg, 2022; Fedorenko et al., 2016). Since cognition, speech and language are distinct, yet interacting features, it is crucial to assess these skills by using standardised test instruments to grasp the full spectrum of features associated with genetic disorders (Chenausky & Tager-Flusberg, 2022). Deep phenotyping of both shared and unique neurodevelopmental and behavioural features has important clinical value, as it often results in better diagnostic trajectories, enhanced prognostic counselling and improved management and therapeutic interventions (Grayton

et al., 2012; Morison et al., 2023; Toriello, 2011). Details about specific approaches will be discussed in the following paragraphs.

1.3.1 Categorical versus dimensional approach

The utilisation of a diagnostic taxonomy clearly offers multiple benefits, reducing uncertainty and enhancing the communication of findings. Categories also play a crucial role in determining eligibility for specific therapeutic interventions and reimbursement of services (Kamphuis & Noordhof, 2009; Nicholls, 2018). However, multiple researchers argue that the traditional categorical diagnoses and classifications have proven to be inadequate for NDDs due to unclear boundaries between disorders, nuanced differences within a category and frequent comorbidity (Morris-Rosendahl & Crocq, 2020; Van Herwegen et al., 2015). Categorical thinking prompts us to simplify complex concepts by attributing them to only one or a few specific variables (Nicholls, 2018).

Therefore, contemporary diagnostic and research approaches advocate for a more dimensional perspective (Jacquemont et al., 2022; Sanders et al., 2019). A dimensional approach involves placing symptoms on a spectrum, focusing on both the severity and impact of symptoms rather than merely their presence (Aftab & Ryznar, 2021; Finucane et al., 2016). Although the DSM-5 attempts to address this by introducing dimensional severity scales for clustered symptoms in specific disorders, it still predominantly adheres to categorical classification (Kim & State, 2014; Vanheule, 2017; Vanopstal et al., 2023). Dimensional assessments require the consideration of asking questions about what, where and how impairments may impact individuals' lives. It is important to characterise strengths and weaknesses, goals and aspirations, support systems and needs of individuals and to track the evolving presentations of their clinical features over the course of life and development (Nicholls, 2018). Moreover, it has been proposed that when treating phenotypic measures as quantitative traits instead of diagnostic categories, the penetrance (i.e. the proportion of individuals with a CNV who also demonstrate phenotypic features, see 1.4.4) of most neurodevelopmental variants would be close to complete. Consequently, it could be more insightful to view the influence of CNVs as potentially "shifting" a particular phenotype or trait from a baseline value determined by a certain genotype, rather than affirming that a CNV leads to a specific disorder in a specific proportion of carriers, as shown in Figure 1.4 (Cable et al., 2021).

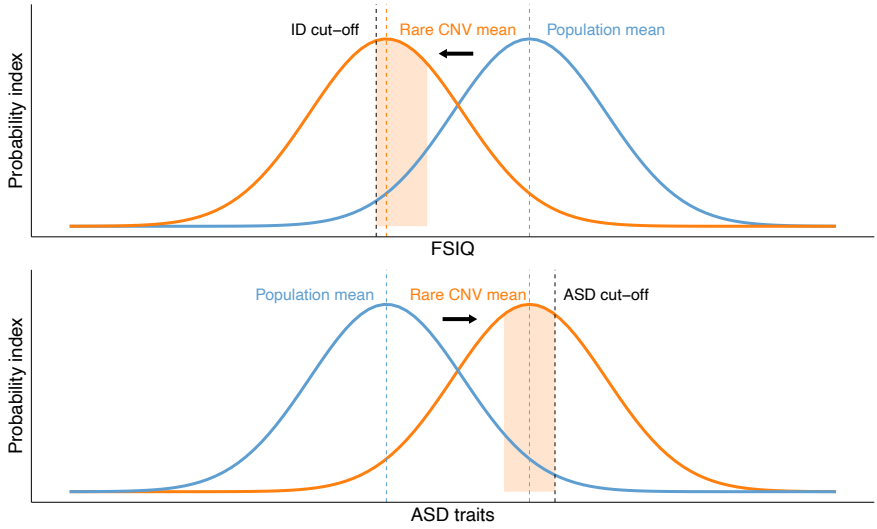


Figure 1.4 – Theoretical depiction effect of rare CNVs on FSIQ and ASD traits. Rare, large effect CNVs such as 22q11.2DS shift the distribution of quantitative traits, such as Full-scale intellectual quotient (FSIQ; top) or ASD traits (bottom). Higher rates of individuals with 22q11.2DS qualify for diagnoses, although many of those who do not meet full diagnostic criteria still exhibit significant symptoms (the orange zone refers to sub-threshold individuals). Consequently, the principle of penetrance with binary diagnostics fails to adequately convey the genuine impact of the CNV and the alteration in the distributions of the quantitative traits. Figure adapted from Cable et al., 2021.

1.3.2 Direct versus indirect assessments

Indirect methods involve collecting data through questionnaires or reports completed by informants, such as parents or teachers. Parental surveys represent a useful initial tool for gathering insights into neurodevelopmental and behavioural features of children with recurrent CNVs, as parents observe their children in multiple natural settings (Bennetts et al., 2016; Bishop & McDonald, 2009; Brown et al., 2020; Garibaldi et al., 2021). Nevertheless, the use of indirect methods introduces potential biases, as questions can be interpreted in various ways and children may perceive difficulties different than their parents (Bennetts et al., 2016; Van Roy et al., 2010). For instance, parents with lower educational levels may encounter challenges in accurately interpreting and responding to survey questions. To mitigate these limitations, indirect instruments should be complemented by direct methods, employing in-person assessments with standardised and age-appropriate test instruments and assessments (Bennetts et al., 2016; Swillen, 2024).

1.3.3 Between-group versus within-group study designs

Dykens and Hodapp (2007) stated that individuals with a genetic condition are more likely to demonstrate some behavioural or neurodevelopmental features than others without this condition. This definition of increased probabilities encourages both between-group and within-group study designs. Between-group comparisons enable researchers to investigate the degree to which syndromic behaviours are unique or shared. Individuals with a genetic condition may be compared to individuals without the genetic condition or with a different genetic condition. Regardless of whether these behaviours are distinctive or shared, they usually exhibit individual differences, and this variability is central to most within-group studies, which focus on describing characteristic features, individual variability across these behaviours and potential genetic, neurologic, and other causes of within-syndrome variability (Dykens & Hodapp, 2007; Karmiloff-Smith et al., 2016).

An interesting approach to integrating both between-group and within-group analyses, is the three-tiered method proposed by Olsson (2005), which is used in the current dissertation. In smaller groups or those with substantial intra-group heterogeneity, it could be useful to complement traditional statistical and quantitative analyses with qualitative and descriptive methods. In a three-tiered method, outcomes are analysed and interpreted from three different perspectives: the group, subgroup and individual level. At the group level, statistical analyses are conducted to determine overall group differences, aligning with the dimensional approach and between-group study design. At the subgroup or intermediate level, researchers compare the proportion of individuals with clinical scores, based on a certain cut-off. This approach aligns more with a categorical view and within-group study design. Subgroup comparisons help identify whether individual variability impacts the overall outcomes of the group. The emphasis lies on the connection between individuals and the group, with each individual exhibiting varying degrees of conformity to the group pattern. At the third or individual level, notable patterns or interesting features of specific individuals are further explored to look for typical or atypical characteristics in comparison to the group (Olsson, 2005).

1.3.4 Cross-sectional versus longitudinal approach

Cross-sectional research provides a snapshot of the phenotypic features prevalent within a specific age range, allowing researchers to quickly identify common characteristics associated with the CNV (Karmiloff-Smith, 2012a; Van

Herwegen et al., 2015; Wang & Cheng, 2020). Additionally, cross-sectional research can be conducted relatively quickly and with fewer resources compared to longitudinal studies, making it a valuable initial step in exploring the phenotypic spectrum of rare CNVs (Wang & Cheng, 2020). However, cross-sectional research has limitations, particularly in understanding how phenotypes evolve over time (Dykens & Hodapp, 2007; Van Herwegen et al., 2015). Longitudinal research, on the other hand, allows for tracking changes in phenotypic expression and investigating developmental trajectories more comprehensively (Karmiloff-Smith, 2012a, 2012b). Longitudinal studies are essential for understanding the dynamic nature of these features over time (Karmiloff-Smith et al., 2016). An ideal approach for investigating NDDs involves initial cross-sectional designs, followed by longitudinal follow-ups (Van Herwegen et al., 2015).

1.4 Copy number variants (CNVs)

As depicted in Figure 1.5, structural variations can involve loss (deletions) or gain (duplications) of segments of DNA, ranging in size from a few hundreds of base pairs to several megabases (Mb) (Alkan et al., 2011; MacDonald et al., 2014; Zarrei et al., 2015, 2019). Copy number variants (CNVs), defined as gain or loss of at least 1000 consecutive base pairs (1 kb), are highly prevalent in the human genome and contribute significantly to human genetic variability, which refers to both population heterogeneity and genetic disorders (Nowakowska, 2017). Within a single human genome, they may account for a 1.2% difference in comparison to the reference human genome (Nowakowska, 2017; Pang et al., 2010). CNVs may encompass various genes and/or regulatory regions, causing disruption of gene function or altering gene dosage (Shawky, 2014).

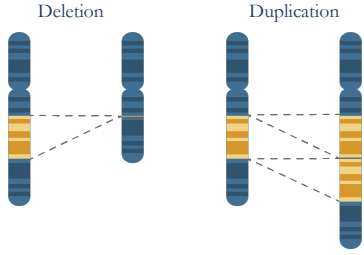


Figure 1.5 – Schematic representation of structural variations of chromosomal segments.
Deletion and duplication on the long arm of the chromosome. Figure adapted from (Hamad, 2023a, 2023b).

Genetic testing for CNV detection includes fluorescence in situ hybridisation (FISH) or multiplex ligation-dependent probe amplification (MLPA) as targeted tools, while conventional karyotyping, chromosomal microarray (CMA including array comparative genomic hybridisation; array CGH) and more recent next-generation sequencing (NGS) methods such as whole exome sequencing (WES) or genome sequencing (GS) are used for genome-wide screening of CNVs (Jacobs et al., 1992; Nowakowska, 2017). The primary complexity related to CNVs lies in determining whether the variant is benign or whether it influences crucial biological functions, leading to the development of disorders (Nowakowska, 2017).

1.4.1 Variant classification categories

To classify CNVs, parental inheritance, available databases, CNV size and the genomic content should be considered. Following several guidelines, each CNV can be categorised into one of the following five fundamental classification categories (Nowakowska, 2017; Riggs et al., 2020):

1. Pathogenic variants: CNVs that are consistently associated with disease/significant clinical phenotypes and have been extensively reported in peer-reviewed studies, acknowledging the presence of reduced penetrance and variable expressivity (see 1.4.4).
2. Likely pathogenic: There is at least 95% certainty that the CNVs will eventually be categorised as disease-causing, although it is still insufficient to assign the CNVs to the pathogenic category.
3. Variants of uncertain significance (VUS): Clinical significance of the CNVs is ambiguous, evidence and peer-reviewed publications are limited. This group encompasses CNVs that cannot be categorised as benign or pathogenic. This category involves gene-containing CNVs with limited information about the dosage sensitivity and the function of the genes (Vermeesch et al., 2012), or gene-poor CNVs that meet the size criteria for documenting.
4. Likely benign: There is at least 95% certainty that the CNVs are not disease-causing, albeit still insufficient to be classified as benign. Examples: CNVs that are frequently observed in the normal population. CNVs that do not statistically significantly differ from healthy controls.
5. Benign variants: CNVs that are not overrepresented in individuals with phenotypic features, have been documented in numerous peer-reviewed studies and are consistently identified (>1%) in the general population (Nowakowska, 2017; Riggs et al., 2020; Savatt & Myers, 2021).

Several features of CNVs support their role in disease pathogenesis. CNVs are considered pathogenic when they contain critical regions of known disease-related chromosomal imbalances or comprise dosage-sensitive genes known to cause a diseased phenotype when mutated. The breakpoints of pathogenic CNVs, referring to a specific location on the chromosome, can be recurrent or unique, as explained below (see 1.4.2). Pathogenic CNVs are considered to have a high effect size and are therefore rare in the general population (figure 1.7; 1.4.3). However, this finding does not preclude more common CNVs from exerting a (small) effect on normal human development as well. In addition, pathogenic CNVs are usually gene-rich, more often involving deletions than duplications, and frequently occurring *de novo* (Shawky, 2014). Indeed, a burden of *de novo* rare variants has been observed in individuals with NDD, leading to a *de novo* paradigm in genetic diagnostics of NDD (Vissers et al., 2010). Diagnostic variant classification pipelines are targeting *de novo* variants, while variants inherited from an apparently unaffected parents are typically disregarded. However, pathogenic variants can be inherited from a parent with a subtle or no expression of the disorder, defined as variable expression or reduced penetrance (see 1.4.4).

1.4.2 Recurrent versus non-recurrent CNVs

Non-allelic homologous recombination (NAHR) is one of the key mechanisms contributing to the formation of recurrent CNVs of similar size with breakpoints clustering in low copy repeats (LCRs) (Hastings et al., 2009; Pös et al., 2021) (see Figure 1.6). In addition, there are CNVs with unique breakpoints, partially overlapping between individuals (Gu et al., 2008; Pös et al., 2021). CNVs with distinct breakpoints sharing a small region of overlap (SRO) may be associated with comparable phenotypes (Pös et al., 2021). CNVs are either inherited from parents or occur *de novo*. CNV breakpoints typically remain the same when being transmitted from a patient to an offspring. *De novo* CNVs occur independently either in a breakpoint-clustering region or at a unique chromosomal location.

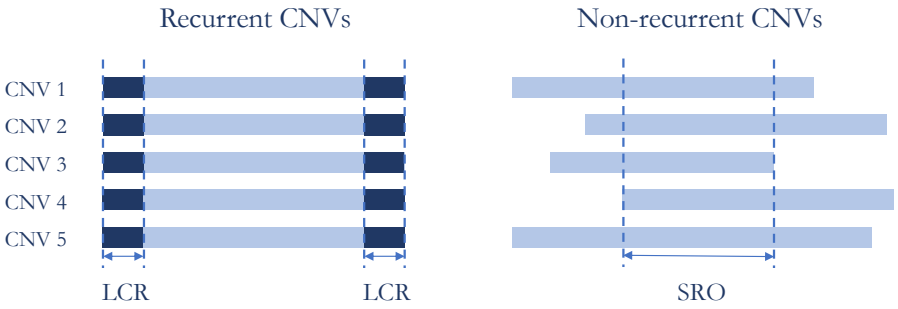


Figure 1.6 – Recurrent versus non-recurrent rearrangements.
Abbreviations: LCR, low copy repeat; SRO, small region of overlap. Figure adapted from Pös et al., 2021.

1.4.3 Rare versus common CNVs

CNVs are seen at diverse rates in the population and may vary depending on ethnic background (J. Li et al., 2009; Yim et al., 2010). A CNV is defined as rare, when the prevalence is below 1% in the general population, in contrast to common or polymorphic CNVs, occurring in the population with a proportion > 1% (Nowakowska, 2017). The human genome is generally considered tolerant to copy number variations (CNVs), as every individual genome contains several common CNVs classified as benign (Mitchell, 2015; Redon et al., 2006; Sebat et al., 2004). Nevertheless, rare CNVs have been recognised as potential risk factors for NDDs (Merner et al., 2015; Mitchell, 2015; Sebat et al., 2004). While individual CNVs are rare, in total, many individuals are affected by CNVs associated with neurodevelopmental delay and ID (Waite et al., 2014). The CNVs detected thus far can explain together a large percentage of cases that were previously unexplained, particularly in conditions such as ASD (>10%) and schizophrenia (>5%) (Mitchell, 2015).

Figure 1.7 depicts the population frequency of the CNV in relation to the degree of pathogenicity. Generally, a negative association exists between the frequency in the population and severity of the impact. Common risk variants typically have subtle effects on illness due to their widespread occurrence. In contrast, rare or low-frequency CNVs are more likely to have larger pathogenic effects, making them valuable for exploring the molecular basis of diseases. Given that CNVs encompass multiple genes, they offer a multi-genic perspective on disease mechanisms, contributing to the comprehension of polygenic disease processes and bridging the gap between monogenic and polygenic risk models (Forrest & Penzes, 2023; Mitchell, 2015).

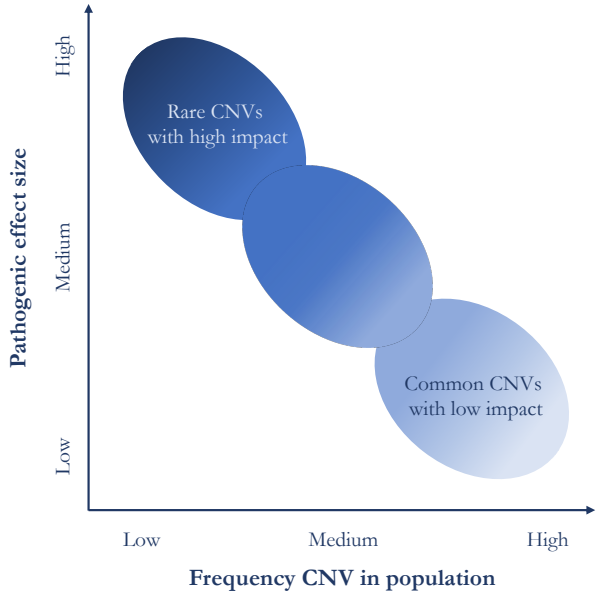


Figure 1.7 – Simplified depiction of the association between population frequency and pathogenic effect size of CNVs related to NDDs.
Figure adapted from Manolio et al., 2009 and Fiksinski, 2020.

1.4.4 Incomplete penetrance, variable expressivity and pleiotropy

The same CNV detected in multiple individuals can result in a broad spectrum of phenotypic features, ranging from clinically asymptomatic to severe clinical symptoms, even across individuals within one family (Bartik et al., 2022; Vergaelen et al., 2015). These concepts are referred to as incomplete penetrance and variable expressivity (see figure 1.8 for theoretical illustration). Penetrance can be defined as the percentage of patients with a certain genotype that also demonstrates the expected clinical phenotype (Cable et al., 2021; Cooper et al., 2013; Kingdom & Wright, 2022). With complete or full penetrance, there is a one-on-one relationship between genotype and phenotype; all individuals who carry the variant are affected. Incomplete or reduced penetrance means that some individuals do not exhibit clinical manifestations, although they share the same genotype. Variable expressivity can be defined as the extent to which a genotype is phenotypically expressed in individuals; the severity of the phenotypic features is variable among individuals (Cable et al., 2021; Shawky, 2014). Both concepts explain why CNVs may be inherited from unaffected parents (Kingdom & Wright, 2022; Shawky, 2014). Several factors may contribute to both concepts, including gene-environmental influences such as having a positive family history

(Davies et al., 2020; A. Moreno-De-Luca et al., 2015; Pizzo et al., 2019) and socioeconomic status, variation in gene expression, common variants and epigenetic changes. A third principle, pleiotropy, can be defined as a specific genotype that is implicated in various phenotypes, generally impacting multiple organ systems (Cable et al., 2021). It is important to consider that most CNVs were established as disease-causing using clinical cohorts with rather small sample sizes. Therefore, the penetrance and expressivity of these genotypes might be overestimated in comparison to their impact on the normal population (Kingdom & Wright, 2022).

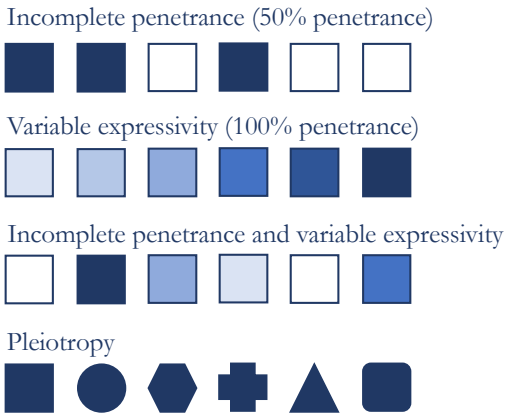


Figure 1.8 – Incomplete penetrance, variable expressivity and pleiotropy.
Each square refers to an individual who carries the same genetic variant, while the shading indicates whether the individual demonstrates the associated phenotypic features or not. The first row is an example of incomplete penetrance, with 50% showing the associated clinical symptoms. The second row is an example of full penetrance, meaning that all individuals are affected, exhibiting a range from mild to severe symptoms. The third row represents incomplete penetrance and variable expressivity, indicated by the genotype that differs in the degree of the symptoms and the penetrance among individuals. The fourth row demonstrates pleiotropy, in which the same variant can affect different phenotypic domains. Figure adapted from Kingdom & Wright, 2022.

The 22q11.2 deletion syndrome (22q11.2DS) is a key illustration of incomplete penetrance, variable expressivity and pleiotropy. However, the conventional inheritance, either occurring *de novo* in a sporadic affected individual or segregating with the disorder when inherited, served as a crucial component for determining its pathogenicity. In contrast, 22q11.2 duplication (22q11.2Dup) and 16p11.2 deletion syndrome/duplication (16p11.2DS/16p11.2Dup) are associated with a higher diversity of phenotypic features and are more frequently inherited from seemingly unaffected parents. Nevertheless, these CNVs are

considered pathogenic based on the increased prevalence of congenital or developmental defects in comparison to control population without these CNVs (Cable et al., 2021; A. Moreno-De-Luca et al., 2013; Nowakowska, 2017; Rosenfeld et al., 2013).

1.4.5 Susceptibility loci

Various CNVs have been identified as susceptibility loci in recent years (Cooper et al., 2011; Girirajan et al., 2011; Girirajan & Eichler, 2010; Kaminsky et al., 2011; Rosenfeld et al., 2013). Susceptibility loci are pathogenic variants posing challenges in genetic counselling. These genetic risk factors exhibit incomplete penetrance, variable expression and/or pleiotropy and are linked to clinical and neurodevelopmental features such as ID, ASD, epilepsy, and psychiatric disorders (Vanakker et al., 2014). The phenotypes associated with such susceptibility CNVs are hard to predict. Detection of one susceptibility CNV can partly solve the genetic aetiology of the phenotype, since the pathogenicity might be influenced by ethnic background, or second hits such as additional CNVs or mutations (Girirajan et al., 2010, 2011; Girirajan & Eichler, 2010; Nowakowska, 2017; Vanakker et al., 2014). Certain CNVs pose a significantly greater risk than others for being more severely affected, such as 16p11.2DS and 22q11.2Dup (Vanakker et al., 2014). Individuals with 22q11.2Dup are estimated to have a penetrance rate of 14-21.9%, while children with proximal 16p11.2DS or 16p11.2Dup have a 31-46.8% or 27.2-34% chance of a clinical phenotype respectively (Kirov et al., 2014; Rosenfeld et al., 2013). However, confidence intervals are wide and varying opinions exist on the validity of these estimates (Benn, 2013; Vanakker et al., 2014).

1.4.6 Gene expression models for genotype-phenotype correlations

Reciprocal CNVs, which are CNVs at the same chromosomal locus, can contribute to unravel causal mechanisms by investigating whether deletions and duplications demonstrate opposing, unique or comparable phenotypic features through additive, dominant or unique models respectively. In an additive model, contrasting phenotypic features are likely to result from expression change in opposite directions for the same genes in the CNV (Deshpande & Weiss, 2018). For example, individuals with 16p11.2 CNVs show opposing head sizes (Steinman et al., 2016). For the dominant model, gene expression changes only contribute to one direction (fewer or more copies), without an impact on the same feature for a change in the other direction. More specific, individuals with

22q11.2DS exhibit late onset overweight or obesity, whereas individuals with 22q11.2Dup have no increased risk of underweight (Deshpande & Weiss, 2018; Zamariolli et al., 2020). In a U-shaped model, decrease or increase of gene expression can result in the same phenotypic features. As an example, both decreased and increased expression of *Tbx1* in mice might cause structural abnormalities such as velopharyngeal deficits and congenital heart disease (Deshpande & Weiss, 2018). In addition, many neurodevelopmental and behavioural phenotypes demonstrate considerable similarities in individuals with deletions and duplications for each reciprocal CNV. For example, ASD is often diagnosed in both deletion and duplication carriers at 16p11.2 and 22q11.2 (Niarchou et al., 2019; Wenger et al., 2016). Phenotypes linked to an additive model more often have a morphological or anatomical nature, whereas neurodevelopmental and behavioural phenotypes are generally shared (U-shaped model) or unique (dominant) between reciprocal CNVs (Deshpande & Weiss, 2018).

1.5 Four copy number variants with elevated risk for neurodevelopmental disorders (NDD-CNVs)

Recurrent CNVs at chromosomal loci 22q11.2 and 16p11.2 are among the most common chromosomal imbalances associated with increased risk for NDDs during life (referred to as NDD-CNVs; S. J. R. A. Chawner et al., 2019), more specific 22q11.2 deletion syndrome/duplication (22q11.2DS – 22q11.2Dup) and 16p11.2 deletion syndrome/duplication (16p11.2DS – 16p11.2Dup). In addition, these NDD-CNVs are the among the most prevalent non-benign copy number variants in patient cohorts and in the general population (Deshpande & Weiss, 2018; Goldenberg, 2018). Some NDD-CNVs have been investigated more broadly than others. Specifically, studies on 22q11.2DS are more common in the literature than studies on 22q11.2Dup, 16p11.2DS and 16p11.2Dup (S. J. Chawner et al., 2021).

1.5.1 22q11.2 copy number variants

Chromosomal region 22q11.2 is rich in low copy repeats (LCRs). These LCRs, starting from proximal LCR-A to distal LCR-H, mediated non-allelic homologous recombination, resulting in recurrent copy number gains or losses. The most prevalent recombination event occurs between LCR22A and LCR22D, giving rise to proximal 22q11.2DS (Online Mendelian Inheritance in Man [OMIM] 188400/192430) and 22q11.2Dup (OMIM 608363), comprising 3

megabases (Mb) (Genomic coordinates (GRCh38): 22:17,400,001-25,500,000) and encompassing approximately 50 coding genes. However, smaller or larger CNVs are also observed (McDermid & Morrow, 2002; McDonald-McGinn et al., 2015; Ou et al., 2008; Portnoï, 2009). The deletion has an estimated prevalence of 1 out of 2148 live births (Blagojevic et al., 2021), whereas the duplication was found to occur 2.5 times more frequently with an estimated prevalence of 1 in 1606 in a Danish population-study (Olsen et al., 2018). In other studies, the duplication was found in 1/1000 to 1/2000 healthy controls (Drmic et al., 2022; Kaminsky et al., 2011; Kendall et al., 2019; Männik et al., 2015; Rees et al., 2014). In clinical cohorts, the prevalence of the duplication has been estimated to range from 1/300 to 1/527 (G. M. Cooper et al., 2011; Drmic et al., 2022; Grati et al., 2015; Kaminsky et al., 2011; Ou et al., 2008). The deletion occurs *de novo* in about 90% (McDonald-McGinn, 2022), whereas the duplication is inherited in about 70% of cases (Coppinger et al., 2009; Ou et al., 2008; Portnoï, 2009; Wincent et al., 2010; Woodward et al., 2019).

22q11.2 deletion syndrome

The most common medical issues of 22q11.2 are congenital heart defects (CHD, 64-69%), palatal defects (67-68%) such as velopharyngeal insufficiency (VPI, 55%), immune related problems (77%), gastrointestinal problems (68%), endocrine abnormalities (55%), hypotonia, scoliosis, genitourinary defects, short stature, and dysmorphic features (Jackson et al., 2019; McDonald-McGinn et al., 2015, 2020, 2022). Of the four NDD-CNVs, only in 22q11.2DS speech-language, behaviour and development have been extensively studied. Remarkably, speech-language difficulties are observed in nearly all children with 22q11.2DS, marking this as a hallmark feature during early childhood. Receptive and expressive language skills are both affected: children may experience deficits in language form (phonology, morphology, syntax), content (semantics, lexical access) or language use (pragmatics). Speech problems involve hypernasality, speech motor delays or speech sound disorders such as childhood apraxia of speech (CAS) or dysarthria (Solot et al., 2019; Van Den Heuvel, Manders, et al., 2018). Most individuals with 22q11.2DS exhibit intellectual skills in the borderline range (FSIQ 70-85) or mild ID (FSIQ 55-69), with a mean of 70 (Campbell et al., 2022; De Smedt et al., 2007; Duijff et al., 2012; Fiksinski, Bearden, et al., 2022; Fiksinski, Heung, et al., 2022; Klaassen et al., 2016; Swillen et al., 2018) The neurodevelopmental and behavioural phenotype is further characterised by delayed motor development and motor deficits, learning disorders, impulsivity, inattention, impaired social relationships,

and NDD diagnoses of ASD, ADHD, anxiety and mood disorders, psychosis and schizophrenia (Fiksinski et al., 2018; Schneider et al., 2014; Swillen & McDonald-McGinn, 2015). The question arises as to whether a duplication at the same locus results in comparable effects on language, speech, behavioural and cognitive skills.

22q11.2 duplication

In general, the 22q11.2Dup is thought to exhibit phenotypic traits similar to those of 22q11.2DS, albeit at lower frequencies and with a less pronounced impact. The partial phenotypic overlap between deletions and duplications in the 22q11.2 region likely results from an ascertainment bias, as 22q11.2Dup has often been diagnosed by FISH in patients with a 22q11.2DS phenotype. Features of 22q11.2DS represent only a small part of the phenotypic spectrum of 22q11.2Dup (Van Campenhout et al., 2012; Verbesselt et al., 2022; Yobb et al., 2005). The 22q11.2Dup is associated with even more variability, which is in combination with its more recent discovery probably leading to reduced rates of genetic testing and leaving many individuals undiagnosed (Edelmann et al., 1999; Ensenauer et al., 2003; Lin et al., 2020; Ou et al., 2008; Portnoï, 2009; Van Campenhout et al., 2012). As a consequence, and due to the subset of individuals exhibiting a near-normal phenotype, individuals with 22q11.2Dup are remarkably less present in the literature, and studies on the phenotype are rather fragmentary (Kortanek et al., 2022). The medical phenotype may involve CHD, palatal defects such as VPI, short stature, genitourinary defects, dysmorphic features, muscular hypotonia, nutritional and sensory problems such as hearing or visual impairments (Bartik et al., 2022; Butensky et al., 2021; Ou et al., 2008; Portnoï, 2009; Van Campenhout et al., 2012; Wenger et al., 2016; Yu et al., 2019).

Multiple case reports and studies in clinically ascertained cohorts have highlighted delays or impairments in speech or language, but none have systematically characterised these observed impairments using standardised methods (Bartik et al., 2022; Courtens et al., 2008; Lo-Castro et al., 2009; Ou et al., 2008; Soysal et al., 2011; Van Campenhout et al., 2012; Yu et al., 2019). Cognitive profiles of individuals with 22q11.2 were reported to be in the low average (FSIQ 85-100) to borderline range (FSIQ 70-85) (S. J. R. A. Chawner et al., 2021; Drmic et al., 2022; Jacquemont et al., 2022; Jalbrzikowski et al., 2022; Lin et al., 2020; Modasi et al., 2023; Modenato, Martin-Brevet, et al., 2021). The neurodevelopmental and behavioural phenotype may further include motor delays/impairments, behavioural problems, anxiety and NDDs including ASD

and ADHD (Bartik et al., 2022; S. J. R. A. Chawner et al., 2021; Drmic et al., 2022; Lin et al., 2020; Olsen et al., 2018; Portnoï, 2009; Van Campenhout et al., 2012; Wenger et al., 2016; Yu et al., 2019; Zhang et al., 2021). Wenger et al. (2016) characterised the neuropsychiatric functioning and estimated the prevalence of ASD on 14-25%. Further characterisation of the neurodevelopmental and behavioural traits is still required (Van Campenhout et al., 2012; Yu et al., 2019).

1.5.2 16p11.2 copy number variants

16p11.2 CNVs, in specific proximal 16p11.2DS (OMIM 611913) and 16p11.2Dup (OMIM 614671), arise from a non-allelic homologous recombination between breakpoints (BP) 4 and 5 at chromosomal locus 16p11.2 resulting in a deletion or duplication with a common size of 593 kilobase (kb), comprising approximately 29 genes (Genomic coordinates (GRCh38): 16:28,500,001-35,300,000). Reciprocal BP4-BP5 deletions and duplications at 16p11.2 have an estimated population prevalence of about 1/2000 and 1/1100 respectively (Chung et al., 2021; Männik et al., 2015). Another predictive algorithm estimated deletions to affect 1/3021 live births, and duplications 1/4216 (Gillentine et al., 2018; Rein & Yan, 2020). Both variants are two of the most common genetic aetiologies of ASD, explaining 0.5-1% of cases with ASD (Fernandez et al., 2010; Hudac et al., 2020; Jacquemont et al., 2011; R. A. Kumar et al., 2008; Marshall et al., 2008; Rosenfeld et al., 2010; Sebat et al., 2007; Walsh & Bracken, 2011; Weiss et al., 2008). The deletion occurs *de novo* in about 60-93%, whereas the duplication is inherited in about 70-84% of cases (Chung et al., 2021; D'Angelo et al., 2016; Green Snyder et al., 2016; Niarchou et al., 2019; Rein & Yan, 2020; Rosenfeld et al., 2010; Steinman et al., 2016; Taylor et al., 2021, 2023; Zufferey et al., 2012). Both variants appear to be mostly maternally inherited (Rein & Yan, 2020).

16p11.2 deletion syndrome

The medical phenotype of 16p11.2DS might implicate overweight or obesity (75%), seizures/epilepsy (20-27%), increased head circumference and macrocephaly (17%), hearing impairment (<11%), and paroxysmal kinesigenic dyskinesia (<9%) (Chung et al., 2021; D'Angelo et al., 2016; El Achkar et al., 2022; Gill et al., 2014; Jacquemont et al., 2011; W. Li et al., 2018; Oliva-Teles et al., 2020; Qureshi et al., 2014; Steinman et al., 2016; Taylor et al., 2021; Zufferey et al., 2012). Congenital abnormalities are reported in 21-58% of the cases, of whom the majority has one isolated medical issue, with vertebral anomalies being

the most observed anomaly (20%) and CHD reported in 6% (Al-Kateb et al., 2014; Chung et al., 2021; Liu et al., 2023; Taylor et al., 202; Zufferey et al., 2012).

A variety of speech and/or language impairments have been associated with 16p11.2DS (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; Rosenfeld et al., 2010; Shinawi et al., 2010). Language disorders (LD; 41-83%) and speech sound disorders (SSD; 50-89%) are reported to be hallmark features of the 16p11.2DS (Chung et al., 2021; S. H. Kim et al., 2020; Mei et al., 2018; Taylor et al., 2021). Using a comprehensive standardised test battery, Mei et al. (2018) concluded that 83% (33/40) of children and adolescents (2.11-18.0 years) had an LD with average core language scores more than two standard deviations (*SD*) below the population mean (Mei et al., 2018), while neuroimaging studies based on the Simons VIP cohort (The Simons VIP Consortium, 2012) found core language scores 1.5 *SD* below the population mean (Ahtam et al., 2019; Berman et al., 2015; Blackmon et al., 2018; Matsuzaki et al., 2020). Deficits were observed across several language components, including morphology, syntax, and semantics. Both expressive and receptive abilities were affected (Hanson et al., 2015; Mei et al., 2018), whereas other studies reported slightly worse expressive than receptive language skills (Ahtam et al., 2019; Blackmon et al., 2018; Hanson et al., 2010). Using both direct and indirect measures, Kim et al. (2020) ascertained syntactic delays in 78-84% and pragmatic-semantic delays in 63%-98% of individuals with 16p11.2DS (2.0-20.10 years) depending on the used instrument. They concluded that language impairments persist after controlling for ASD and ID. It is speculated that delays in speech and language development may contribute to behavioural problems, but this relation is not well understood and requires further investigation (Taylor et al., 2021). Regarding speech impairments, Mei et al. (2018) concluded that the majority of children (77%) met criteria for CAS, confirming the association with CAS indicated by previous studies (Fedorenko et al., 2016; Raca et al., 2013). In addition, a significant subgroup demonstrated phonetic or phonological errors, dysarthria, and minimal verbal output. The fact that motor speech control is affected in 16p11.2DS, suggests that language and motor impairments might be associated (Demopoulos et al., 2018; Steinman et al., 2016).

Cognitive abilities vary widely, spanning from an average FSIQ to ID, with average FSIQ typically falling within the borderline range (FSIQ 70-84) and up to 70% having ID (S. J. R. A. Chawner et al., 2021; Hanson et al., 2015;

Hippolyte et al., 2016; Jutla et al., 2020; S. H. Kim et al., 2020; Mei et al., 2018; Modenato, Kumar, et al., 2021; A. Moreno-De-Luca et al., 2015; Oliva-Teles et al., 2020). FSIQ scores of probands with 16p11.2DS are, on average, two *SD* below those of their unaffected first-degree relatives (Hanson et al., 2015; Hippolyte et al., 2016; Zufferey et al., 2012). Verbal and non-verbal IQ (VIQ – NVIQ) span a comparable range, with VIQ scores generally slightly lower (S. J. R. A. Chawner et al., 2021; D'Angelo et al., 2016; Hanson et al., 2015; Hudac et al., 2020; Jacquemont et al., 2022; A. Moreno-De-Luca et al., 2015; Mortillo & Mulle, 2021). In line with these results, Zufferey et al. (2012) found significantly lower VIQ than NVIQ. Additionally, there was a tendency towards decreased FSIQ in individuals with inherited 16p11.2DS (FSIQ 74) in comparison to individuals with *de novo* deletions (FSIQ 83), which is in line with other studies (D'Angelo et al., 2016; Gill et al., 2014; Hanson et al., 2015). A longitudinal study in individuals with 16p11.2DS between 6 months and 8 years found improvements in VIQ over time (Bernier et al., 2017). Longitudinal profiles beyond the age of eight and detailed cognitive profiles remain to be explored.

The neurodevelopmental phenotype further involves psychiatric/behavioural issues (>90%), motor delays (50-57%), problems with gross and fine motor skills and motor coordination difficulties (60%) (Bijlsma et al., 2009; Chung et al., 2021; Deshpande & Weiss, 2018; Goldman et al., 2019; Hanson et al., 2015; Hippolyte et al., 2016; Rein & Yan, 2020; Shinawi et al., 2010; Steinman et al., 2016). Individuals with 16p11.2DS demonstrate significantly more behavioural issues compared to their unaffected siblings, with relatively more internalising than externalising behaviours, referring to anxiety, mood related problems and sleep issues (Hanson et al., 2015). Behavioural impairments tend to increase with increasing age, as indicated by the declining social, daily living and motor skills and increasing internalising behaviours in the study of Bernier et al. (2017). Diagnoses of at least one NDD were reported in 48-93%, including ASD in 16-25%, developmental coordination disorder (DCD) in 32-58%, ADHD in 29%, and anxiety, psychotic symptoms and affective problems (Degenhardt et al., 2012; Deshpande & Weiss, 2018; Hanson et al., 2015; Niarchou et al., 2019; Rein & Yan, 2020; Taylor et al., 2021). ASD is diagnosed in an important subgroup of individuals with 16p11.2DS (20-25%) (Bijlsma et al., 2009; S. J. R. A. Chawner et al., 2019; Chung et al., 2021; D'Angelo et al., 2016; Deshpande & Weiss, 2018; Fedorenko et al., 2016; Goldenberg, 2018; Goldman et al., 2019; S. H. Kim et al., 2020; Maillard et al., 2015; A. Moreno-De-Luca et al., 2015; Niarchou et al., 2019; Shinawi et al., 2010; Steinman et al., 2016; Taylor et al., 2021; Zufferey et al., 2012). Although not all individuals with

the 16p11.2DS meet diagnostic criteria for ASD, nearly all exhibit certain behavioural characteristics associated with ASD such as restricted interests, repetitive behaviours, and social communicative difficulties, resulting in a significant shift in social responsiveness compared to intrafamilial controls (Chung et al., 2021; Fetit et al., 2020; Hanson et al., 2015; A. Moreno-De-Luca et al., 2015; Taylor et al., 2021; Zufferey et al., 2012).

16p11.2 duplication

In general, individuals with 16p11.2Dup demonstrate a variable phenotype (D'Angelo et al., 2016; Green Snyder et al., 2016; Taylor et al., 2021). Reciprocal to deletion carriers, individuals with duplications tend to have smaller head circumference or microcephaly (17-23%), and underweight or lower body mass index (BMI) (Chung et al., 2021; D'Angelo et al., 2016; Deshpande & Weiss, 2018; Goldenberg, 2018; Jacquemont et al., 2011; Oliva-Teles et al., 2020; Qureshi et al., 2014; Rein & Yan, 2020; Shinawi et al., 2010; Zufferey et al., 2012). Similarly to the deletion, the medical phenotype of 16p11.2Dup is further characterised by epilepsy and seizures in 15-29% (D'Angelo et al., 2016; Deshpande & Weiss, 2018; El Achkar et al., 2022; Goldenberg, 2018; Knoll et al., 2018; Reinthaler et al., 2014; Shinawi et al., 2010; Steinman et al., 2016; Taylor et al., 2021). Dysmorphic features or congenital anomalies were noted in 16-29% of individuals with 16p11.2Dup (Rein & Yan, 2020).

Regarding language profiles, Kim et al. (2020) ascertained syntactic delays in 46-56% and pragmatic-semantic delays in 48-96% of individuals with 16p11.2Dup (2.0-23.5 years) using both direct and indirect instruments (S. H. Kim et al., 2020). Two studies found average language scores one *SD* below the population mean (Blackmon et al., 2018; Green Snyder et al., 2016) with speech-language disorders found in 32% (20/62) (Green Snyder et al., 2016). Steinman et al. (2016) mentioned speech sound errors in 46% of children with 16p11.2Dup, while other studies describe speech-language delays, but in-depth speech and language characterisation is still limited and was mostly done in neuroimaging studies to correlate language outcomes to neuroanatomic structures (Blackmon et al., 2018; D'Angelo et al., 2016; Girirajan et al., 2010; Hippolyte et al., 2016; Maillard et al., 2015; Matsuzaki et al., 2020; Niarchou et al., 2019; Posar & Visconti, 2020; Rosenfeld et al., 2010).

The cognitive profile of 16p11.2Dup is characterised by cognitive delays or ID in up to 40%, with an average FSIQ in the borderline range, which is

approximately one to two *SD* lower than in family members without the duplication depending on the clinical ascertainment (S. J. R. A. Chawner et al., 2021; D'Angelo et al., 2016; Deshpande & Weiss, 2018; Green Snyder et al., 2016; Hippolyte et al., 2016; Jutla et al., 2020; Knoll et al., 2018; Oliva-Teles et al., 2020; Shinawi et al., 2010; Taylor et al., 2023). In contrast to the deletion, several studies found slightly lower NVIQ than VIQ in individuals with 16p11.2Dup (Bernier et al., 2017; Green Snyder et al., 2016; Hippolyte et al., 2016; S. H. Kim et al., 2020; Mortillo & Mulle, 2021). Lower NVIQ scores were associated with lower language skills, ASD diagnosis, social problems and motor impairments (Green Snyder et al., 2016). In a longitudinal study, Bernier et al. (2017) found improvements in VIQ over time.

Other neurodevelopmental and behavioural characteristics include motor delays (60%) and difficulties, autistic traits, and behavioural problems including attention problems and aggressive behaviours with increasing externalising behaviours over time (Bernier et al., 2017; Deshpande & Weiss, 2018; Goldman et al., 2019; Green Snyder et al., 2016; Knoll et al., 2018; Qureshi et al., 2014; Rein & Yan, 2020; Rosenfeld et al., 2010; Shinawi et al., 2010). In the study of Niarchou et al. (2019), 63% of individuals with 16p11.2Dup met criteria for at least one NDD, with ADHD being the most reported diagnosis (in 42%). Duplication carriers had significantly higher rates of ADHD and psychotic symptoms than deletion carriers (Niarchou et al., 2019). Other common NDDs associated with the duplication are ASD in 20-34%, DCD in 47%, anxiety, depression, schizophrenia and bipolar disorder (H. Chang et al., 2017; D'Angelo et al., 2016; Degenhardt et al., 2012; Deshpande & Weiss, 2018; Giaroli et al., 2014; Goldenberg, 2018; Green Snyder et al., 2016; Jutla et al., 2020; Knoll et al., 2018; Maillard et al., 2015; Marshall et al., 2017; McCarthy et al., 2009; Niarchou et al., 2019; Rein & Yan, 2020; Taylor et al., 2021; Zhou et al., 2018).

1.6 The current dissertation

1.6.1 Research objectives

The overall aim of this dissertation is to better delineate and characterise language, cognitive and behavioural profiles in school-aged children with 22q11.2DS, 22q11.2Dup, 16p11.2DS and 16p11.2Dup and investigate whether an association exists between these behavioural and neurodevelopmental areas in the selected NDD-CNVs. Although the selected NDD-CNVs exhibit both shared and distinct clinical features, these are all characterised by a wide and variable phenotype including both medical problems and neurodevelopmental

difficulties. In the existing literature, systematic, protocol-driven research on language, behavioural and cognitive difficulties is rather fragmentary and no clear distinction has been made between speech and language impairments, particularly in 22q11.2Dup and 16p11.2 CNVs. Language and speech are important research topics because of their relation to and comorbidity with behaviour, cognition, academic performance, daily communication, socio-emotional development and quality of life (Van Agt et al., 2011; Vyshedskiy et al., 2017). Furthermore, previous studies in individuals with 16p11.2 CNVs have primarily relied on data from the Simons VIP cohort or have lacked appropriate comparison groups. In addition, no research exists on the association between speech-language problems and comorbid cognitive impairments and behavioural disorders in these NDD-CNVs. Deep phenotyping of language, cognition and behaviour is important for understanding the nature, occurrence and severity of neurodevelopmental difficulties associated with these four CNVs. From a clinical perspective, this understanding is crucial for informing healthcare professionals, and guiding neurodevelopmental follow-up and intervention strategies aimed at reducing the potential long-term effects of these difficulties. From a theoretical perspective, studying specific CNVs provides a more homogeneous basis for examining phenotypes compared to studying children with neurodevelopmental difficulties and disorders without a known genetic variant or cause. We hypothesise that, compared to the general population (norm groups) and unaffected siblings, children with the selected NDD-CNVs demonstrate delayed speech-language acquisition, speech-language impairments, learning problems and cognitive delays/deficits, problems with social responsiveness, and externalising and/or internalising behavioural difficulties. Furthermore, we assume a relation exists between speech-language impairments and behavioural difficulties.

Figure 1.9 depicts an overview of the different domains and subdomains of the neurodevelopmental and behavioural phenotype assessed in the different studies. The following research questions are addressed in six separate studies:

Chapter 2 (Study 1) - Which clinical, behavioural and neurodevelopmental features do patients with the 22q11.2Dup show? Which cross-sectional cognitive profiles and longitudinal cognitive trajectories do children with 22q11.2Dup demonstrate?

Chapter 3 (Study 2) - Do parents report specific social-communicative challenges in children with 22q11.2Dup in comparison to their unaffected siblings, age-matched children with 22q11.2DS and the normative sample?

Chapter 4 (Study 3) - Which language profiles do children with 22q11.2Dup demonstrate compared to age-matched children with 22q11.2DS or the normative sample?

Chapter 5 (Study 4) - Do parents report specific social-communicative and behavioural challenges in children with 16p11.2 CNVs in comparison to children with the reciprocal CNV and the normative sample? Are communicative impairments associated with behavioural manifestations in the selected CNVs?

Chapter 6 (Study 5) - Do children with 16p11.2DS show delayed developmental milestones, compared to the normative sample? Which cross-sectional cognitive profiles and longitudinal cognitive trajectories do children with 16p11.2DS show?

Chapter 7 (Study 6) - Which language profiles do children with 16p11.2 CNVs demonstrate compared to the normative sample and unaffected siblings of children with 16p11.2DS?

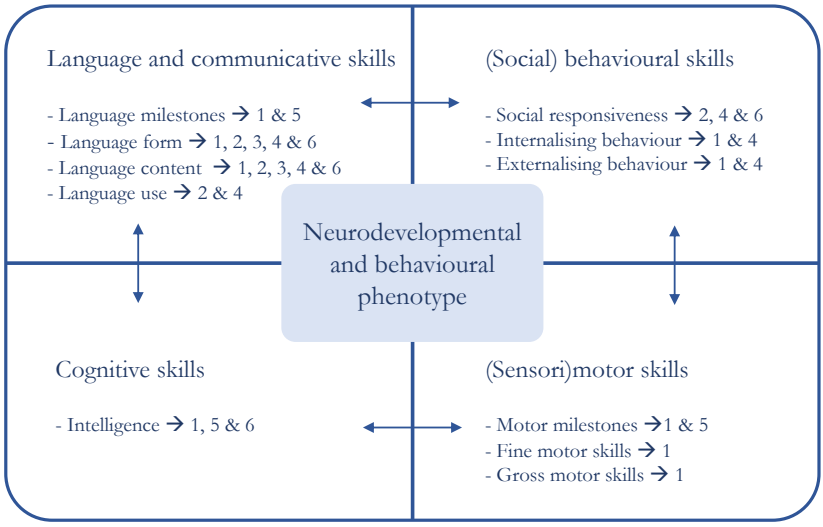


Figure 1.9 – Overview of the four core domains of the neurodevelopmental and behavioural phenotype with their respective subdomains assessed in the different studies (1-6).

1.6.2 Participants

In total, 173 different participants were included across six different studies, including 154 with NDD-CNVs (n = 34 with 22q11.2DS, n = 50 with 22q11.2Dup, n = 49 with 16p11.2DS and n = 21 with 16p11.2Dup) and 19 siblings without CNV. For each study, an overview of the exact sample sizes is provided in Table 1 and Supplementary Table 1. Many individuals participated in several studies; the overlap between studies is shown in supplementary figure 1.1.

For the first study, we included all participants known with 22q11.2Dup at the genetic clinic of the University Hospitals Leuven, which were in total 28 participants with 22q11.2Dup aged 4.1 and 56.7 years with a median age of 17.11 years. For studies 2 to 6, we included school-aged children between 5-17 years with an aimed sample size of 20 children per CNV to reach sufficient statistical power. The school age range of 5-17 years is a critical period for development and learning, providing an opportune time for support and therapy. This period is marked by significant growth as children transition from early childhood to adolescence. With language skills sufficiently developed, this age range allows for comprehensive examination and comparison of communicative skills, even among children with NDDs, including language disorders. Compared to younger ages, there is less variability in language development within this age range, offering a stable foundation for research and intervention (Reed, 2018).

Participants with CNVs were predominantly recruited through the Centre for Human Genetics at University Hospitals Leuven, supplemented by participants recruited through other Centres for Human Genetics across Flanders and the Netherlands. Data for studies 2-6 were prospectively collected during consultations at the hospital or/and home visits from 2012 to 2023. To maximise time efficiency, data collection for different studies was combined. Study 3 and study 4 were multi-site studies to increase the sample sizes and statistical power. Participants in study 3 were also recruited through the Children's Hospital of Philadelphia (CHOP) in the USA, while participants in study 4 were additionally recruited through the Geisinger Autism & Developmental Medicine Institute (Geisinger ADMI), Washington University in St. Louis and University of Washington.

The first control group consisted of unaffected siblings of children with CNVs (see table 1.1), providing insight into genetic and environmental background influences that might modulate cognitive, neurodevelopmental,

behavioural and psychiatric outcomes in children with CNVs (Flynn, 2016; A. Moreno-De-Luca et al., 2015). Second, cross-CNV comparisons were performed to look for syndrome-specific features (Mervis, 2004). Finally, profiles of children with CNVs and their siblings were compared to those of typically developing peers in the normal population through norm group scores (referred to as the normative sample).

Using a genetic-first approach, all participants with CNVs had a laboratory confirmed diagnosis based on chromosomal microarray (array CGH), multiplex ligation-dependent probe amplification (MLPA), exome sequencing with CNV calling or fluorescent in situ hybridisation technique (FISH). All participants with CNVs were index patients diagnosed in clinical settings due to their medical or developmental difficulties or a combination of both. We excluded participants carrying more than one (likely) pathogenic CNV because of the potential clinical impact. Additionally, we excluded participants with CNVs outside of the standard LCR22A-LCR22D or BP4-BP5 region for 22q11.2 and 16p11.2 CNVs respectively given the lack of a minimally overlapping region. Additional exclusion criteria for all participants encompassed: extreme prematurity (i.e., gestational age < 32 weeks), native language other than Dutch/English, and moderate to severe hearing loss (≥ 35 dB HL) given the established impact on language abilities (Barre et al., 2011; Crosbie et al., 2011; Cummins, 2000; Kohnert et al., 2021; Lieu et al., 2020). Participants with comorbid NDDs such as ADHD, ASD and cerebral visual impairment (CVI), were not excluded from the sample due to the increased comorbidity.

1.6.3 Protocol

The research project and protocol were approved by the Ethics Committee Research (EC Research) of University Hospitals Leuven (UZ Leuven) on the 13th of February 2020 (S62997) and 26th of March 2021 (S54484). The collaborative studies were conducted in accordance with and approved by the Institutional Review Board at the Children's Hospital of Philadelphia (CHOP) and the Geisinger Autism and Developmental Medicine Institute (ADMI). Study-specific details (see Table 2) and the exact execution of each protocol can be found in the respective chapters.

Table 1.1 – Details target and control groups, and research designs across six studies

	Chapter 2 (S1)	**Chapter 3 (S2)**	**Chapter 4 (S3)**	**Chapter 5 (S4)**	**Chapter 6 (S5)**	**Chapter 7 (S6)**
CNV loci	22q11.2Dup	22q11.2Dup 22q11.2DS	22q11.2Dup 22q11.2DS	16p11.2DS 16p11.2Dup	16p11.2DS	16p11.2Dup 16p11.2DS
Age	4-56 years	6-16 years	6-15 years	6-17 years	5-16 years	5-16 years
Control group/ comparisons	/	Norm group Cross-CNV Siblings	Norm group Cross-CNV	Norm group	Norm group	Norm group Cross-CNV Siblings
Recruitment site	Leuven	Leuven	Leuven + USA	Leuven + USA	Leuven	Leuven
Total n	28	49	58	68	24	41
Domain	Language Cognition Behaviour Motor	Language Behaviour	Language	Language Cognition Behaviour	Language Cognition Motor	Language Cognition Behaviour
Retrospective/prospective	Retrospective	Prospective	Both	Prospective	Both	Prospective
Indirect/direct	Both	Indirect	Direct	Indirect	Both	Both
Cross-sectional/longitudinal	Both	Cross-sectional	Cross-sectional	Cross-sectional	Both	Cross-sectional
Categorical/dimensional	Both	Both	Both	Both	Both	Both
Confounding factors	Inheritance	Inheritance, sex, country	Inheritance, sex, country, SES, medical, NDD	Inheritance, country, SES, age	Inheritance, sex, age, NDD	Inheritance, sex, NDD

Chapter 2 – Retrospective chart study

For the study in Chapter 2, we performed a detailed retrospective analysis of all digital clinical files of participants with 22q11.2Dup, known at the University Hospitals Leuven (UZ Leuven), whose data had been recorded for various purposes, such as diagnostics or medical follow-up. Of the initial 37 patients, nine were excluded based on the presence of additional CNVs with potential clinical impact (n = 4), atypical breakpoints (n = 1) or breakpoints located distal to the typically 3.1 Mb duplicated region (n = 4). The digital clinical files comprised information on genomics, medical history, clinical phenotype, early neurodevelopmental milestones, and behavioural features. Information regarding developmental milestones, assessing the timely achievement of milestones in various neurodevelopmental areas, relied on clinical follow-up or developmental history reported by parents. Neurodevelopmental and behavioural difficulties were defined as characteristics that were recorded in medical files by (para)medical personnel and were evaluated using screening assessments and questionnaires. Only parents and teachers were included as informants. NDDs were diagnosed by a multidisciplinary team, comprising healthcare professionals, paediatric neurologists, or child and adolescent psychiatrists, through standardised psychiatric interviews and diagnostic instruments, according to the DSM-5 (American Psychiatric Association, 2013). In 19 participants, standardised intelligence instruments were administered. A subset of 11 participants had undergone formal cognitive testing at two or three different time points.

Chapters 3 and 5 - Indirect assessments of social-communicative and behavioural skills

For the studies in Chapters 3 and 5, deep phenotyping was performed in an indirect manner. Indirect assessments were useful since parents play a significant role as source of information. The online platform Qualtrics was used to provide and complete questionnaires. Regarding communication, the Children's Communication Checklist – second version (CCC-2-NL) (Geurts, 2007) and an anamnestic questionnaire were used. The CCC-2-NL is a standardised parental questionnaire on social-communication difficulties. This indirect instrument allows us to tap into the broad spectrum of daily communicative situations, referring to speech, structural, semantic and pragmatic language abilities and social skills (Bishop, 1998; Norbury et al., 2004). An anamnestic questionnaire was developed to obtain information on

developmental history, achievement of speech-language milestones and familial history with focus on the presence of learning disabilities, speech-language impairments and neurodevelopmental and behavioural difficulties.

Behaviour and social-emotional development were measured using two standardised parental questionnaires, the Child Behaviour Checklist (CBCL 6-18) (Achenbach & Rescorla, 2001; Verhulst & Van der Ende, 2013) and the Social Responsiveness Scales – Second edition (SRS-2) (Constantino & Gruber, 2012; Roeyers et al., 2015). The CBCL is one of the most universally used questionnaires in the assessment of behavioural and emotional difficulties, recognising problems across eight syndrome scales and resulting in externalising and internalising problems (Noterdaeme & Amorosa, 1999; Rossi & Giacheti, 2017). The SRS-2 measures deficits in social behaviour associated with ASD, consisting of the subscales Social Awareness, Social Cognition, Social Communication, Social Motivation and Restricted Interests and Repetitive Behaviour (Bruni, 2014).

Chapters 4, 6 and 7 - Direct assessment of language and cognition

For the studies in Chapters 4, 6 and 7, the neurodevelopmental domains language and cognition were assessed in a direct way. Direct assessment of children's structural and semantic language skills was fulfilled by administering the Clinical Evaluation of Language Fundamentals-Preschool-Second Edition (CELF-P2-NL) and/or CELF-4-NL (Kort et al., 2010) in Leuven and the CELF-Third, Fourth and Fifth editions in the USA (Paslawski, 2005; Semel et al., 2003; Semel et al., 1995; Wiig et al., 2013). These tests served as measures to assess expressive morphology, expressive and receptive vocabulary and syntax and verbal short-term memory. For study 6, the Peabody Picture Vocabulary Test-Third edition-Dutch version (PPVT-III-NL) was also administered to assess receptive vocabulary (Dunn & Dunn, 1997; Schlichting, 2005).

Information on cognitive abilities of the participants was essential to be able to relate language and communicative outcomes to the cognitive level. If a standardised intelligence test had already been administered in the past year, the available results were used and the test was not administered again. Cognitive assessments were conducted by Master students of Clinical and Educational Psychology under supervision of Prof. A. Swillen. The Wechsler Intelligence Scale for Children – Fifth Edition – Dutch Edition (WISC-V-NL) was used to

measure full-scale intelligence quotient (FSIQ) and five broad cognitive indices (Hendriks et al., 2018; Wechsler, 2014).

1.6.4 Thesis outline

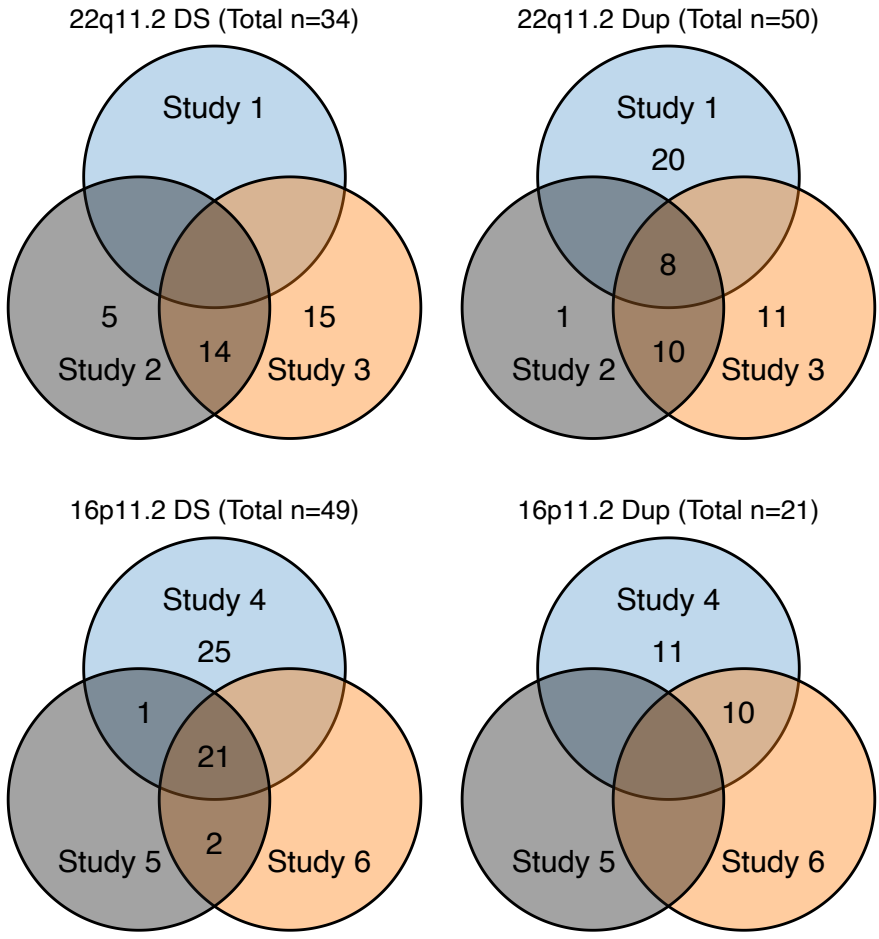
Study 1 to 6 are described respectively in Chapters 2 to 7. **Chapter 2** contains the chart study on medical, neurodevelopmental, behavioural and cognitive features in 22q11.2Dup. **Chapter 3** includes the indirect study on social-communicative abilities in children with 22q11.2Dup compared to their unaffected siblings, age-matched children with 22q11.2DS and the normative sample. **Chapter 4** is the collaborative direct study with CHOP on language profiles in children with 22q11.2Dup in relation to their age-matched children with 22q11.2DS and the normative sample. **Chapter 5** describes the indirect study on the behavioural and social-communicative abilities and the relation between both in children with 16p11.2 CNVs compared to the normative sample. **Chapter 6** includes the direct study on cognitive profiles and longitudinal cognitive trajectories in children with 16p11.2DS. **Chapter 7** focuses on language profiles of children with 16p11.2 CNVs with cross-CNV and intrafamilial comparisons with unaffected siblings of children with 16p11.2DS, as well as in relation to the normative sample. Study 1 to 4 have been published in international peer reviewed journals, study 5 and 6 are currently under review. The six studies are followed by chapter 8. **Chapter 8** contains the general discussion of the acquired results of the five studies. In this final chapter, we summarise and discuss the results and their clinical implications, link them to future research and finish with an overall conclusion.

Supplementary material

Supplementary Table 1.1 – Sample sizes and recruitment sites across six studies.

	22q11.2DS **Total *N* = 34**		**22q11.2Dup** **Total *N* = 50**		**Siblings of 22q11.2Dup** **Total *N* = 11**	**Total**
Recruitment site	Leuven *N*	USA* *N*	Leuven *N*	USA* *N*	*N*	*N*
Number of participants recruited *N*	23	11	39	11	11	95
Chapter 2 - Study 1: Chart study 22q11.2	/	/	28	/	/	28
Chapter 3 - Study 2: Parent report 22q11.2	19	/	19	/	11	49
Chapter 4 - Study 3: Language 22q11.2	18	11	18	11	/	58
	16p11.2DS **Total *N* = 49**		**16p11.2Dup** **Total *N* = 21**		**Siblings of 16p11.2DS** **Total *N* = 8**	**Total**
Recruitment site	Leuven *N*	USA** *N*	Leuven *N*	USA** *N*	*N*	*N*
Number of participants recruited *N*	26	23	11	10	8	88
Chapter 5 - Study 4: Parent report 16p11.2	24	23	11	10	/	68
Chapter 6 - Study 5: Cognition 16p11.2	24	/	/	/	/	24
Chapter 7 - Study 6: Language 16p11.2	23	/	10	/	8	41

Note. * For 22q11.2 CNVs, USA refers to the Children's Hospital of Philadelphia (CHOP). ** For 16p11.2 CNVS, USA refers to Geisinger Autism & Developmental Medicine Institute, Washington University in St. Louis and University of Washington



Supplementary Figure 1.1 – Overlap of participants across six studies for each CNV group.
Study 1 to 3 at the top with 22q11.2 CNVs and study 4 to 6 at the bottom with 16p11.2 CNVs.

Chapter 2

Chapter 2 - Cross-sectional and longitudinal findings in patients with proximal 22q11.2 duplication: A retrospective chart study

The content of this chapter has been published as: Verbesselt, J., Zink, I., Breckpot, J., & Swillen, A. (2022). Cross-sectional and longitudinal findings in patients with proximal 22q11.2 duplication: A retrospective chart study. *American journal of medical genetics. Part A, 188(1), 46–57. https://doi.org/10.1002/ajmg.a.62487.*

Abstract

Duplications on Chromosome 22q11.2 (22q11.2Dup) are associated with a wide spectrum of physical and neurodevelopmental features. In this chart review, physical, developmental, and behavioural features of 28 patients with 22q11.2Dup (median age = 17.11 years) are reported, and phenotypes of *de novo* and inherited duplications are compared. Common medical anomalies include nutritional problems (57%), failure to thrive (33%), transient hearing impairment (52%), and congenital heart defects (33%). Developmental, speech-language, and motor delay are common in infancy, while attention (64%), learning (60%), and motor problems (52%) are typically reported at primary school age. Attention-deficit/hyperactivity disorders are diagnosed in 44%. Median full-scale intelligence quotient is in the borderline range (IQ 76), with one-fifth of patients having mild intellectual disability. Longitudinal data in 11 patients, with the first assessment at a median age of 5.2 years and the second assessment at a median age of 8.8 years, indicate that almost two-third of patients have a relatively stable cognitive trajectory, whereas one-third show a growing into deficit profile. In patients with *de novo* duplications, there is a trend of more failure to thrive, while more patients with inherited duplications follow special education.

2.1 Introduction

Low copy repeats (LCRs) located in the 22q11.2 region confer risk for copy number variants (CNVs) including the 22q11.2 deletion syndrome (22q11.2DS) or Velocardiofacial syndrome (VCFS) and the 22q11.2 microduplication (22q11.2Dup) syndrome (McDermid & Morrow, 2002). The 22q11.2Dup arises from non-allelic homologous recombinations between LCR22A and LCR22D at chromosomal locus 22q11.2 resulting in an extra copy with a typical size of 3 megabases (Mb), comprising about 40 genes (Firth, 2013). Molecular characterisation demonstrates that smaller or larger duplications (1, 1.5, 4, and 6 Mb) are also common. There seems to be no association between duplication size or location and phenotypic severity (Coppinger et al., 2009; Portnoï, 2009). There is a high rate of familial transmission with the majority of 22q11.2Dups inherited from a parent with a normal or near-normal clinical presentation (Coppinger et al., 2009; Ou et al., 2008; Portnoï, 2009; Wincent et al., 2010; Woodward et al., 2019).

Since 1999 cases with the 22q11.2Dup have been reported (Edelmann et al., 1999), and approximately 350 cases have been published so far. The true prevalence of 22q11.2Dup is hard to establish and probably underestimated due to the difficulty of diagnosis in carriers with a normal phenotype and the limited knowledge on the whole phenotypic spectrum of this CNV (Lo-Castro et al., 2009; Yobb et al., 2005). Most cases have been identified in patients with a phenotype similar to that of individuals with 22q11.2DS. The partial phenotypic overlap between deletions and duplications in the 22q11.2 region is likely due to an ascertainment bias resulting from the diagnosis of 22q11.2Dup by Fluorescence In Situ Hybridisation (FISH) in patients with a VCFS phenotype (Firth, 2013). Features of VCFS represent only a small part of the phenotypic spectrum of 22q11.2Dup (Van Campenhout et al., 2012; Yobb et al., 2005).

As a result of incomplete penetrance and variable expressivity and severity, the 22q11.2Dup encompasses a variety of phenotypes. The physical presentation is characterised by cardiovascular defects, dysmorphic features, growth retardation, muscular hypotonia, cleft lip/palate, velopharyngeal insufficiency, urogenital anomalies, and neurological impairments such as feeding, sensory (hearing impairment and visual problems) and motor problems (Firth, 2013; Woodward et al., 2019; Yu et al., 2019). The neurodevelopmental phenotype includes cognitive impairments, speech and motor delay, behavioural problems, and neurodevelopmental disorders (NDDs) (Firth, 2013; Lin et al.,

2020; Van Campenhout et al., 2012) such as autism spectrum disorders (ASD) with an estimated prevalence of 14%–25% and attention-deficit/hyperactivity disorder (ADHD) (Chawner et al., 2019; Lin et al., 2020; Olsen et al., 2018; Portnoï, 2009; Van Campenhout et al., 2012; Wenger et al., 2016; Yu et al., 2019; Zhang et al., 2021). The aim of the current study is to report on the phenotypic and neurodevelopmental findings of 28 patients diagnosed with the proximal 22q11.2Dup, and to focus on longitudinal data in a small subset in order to further contribute to the phenotypic characterisation of this recurrent CNV. The impact of the inheritance pattern on the phenotype will be investigated by comparing the phenotype of patients with *de novo* and inherited duplications.

2.2 Materials and methods

2.2.1 Editorial policies and ethical considerations

Research and ethics board approval has been obtained. The study protocol was approved by the Institutional Review Board of the University Hospitals Leuven (S52418). Patients were not directly informed, and informed consent was not required.

2.2.2 Patient selection

At the Centre for Human Genetics of UZ Leuven, all 22q11.2Dup diagnoses (by FISH or microarray) until 2018 were included in this retrospective study. Thirty-seven patients with different sizes in the 22q11.2Duplication region were discovered. One patient was excluded because she was carrying a very small, atypical duplication (0.07 Mb) with unique breakpoints proximal to LCR22D (see Figure 2.1). In eight patients (8/37, 22%), an additional rare CNV was detected. Four of them were excluded since this additional CNV may at least partially explain the clinical phenotype (large deletion on Chromosome 4, 15q13.2 deletion, 21q21.1 deletion, and 15q13.2q13.3 deletion).

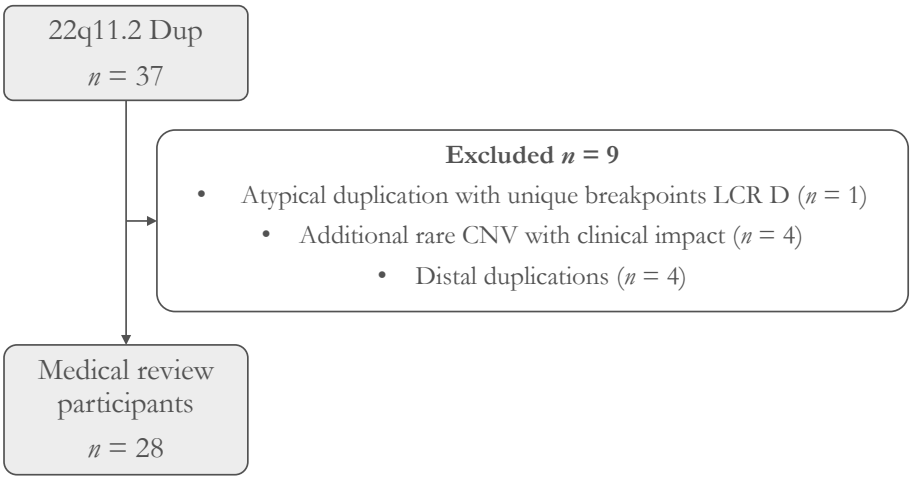


Figure 2.1 – Flow chart for inclusion and exclusion of patients in this study.

The remaining four patients with an additional CNV were included since these CNVs were classified as variants of unknown significance or variants with limited clinical impact, after review of an expert clinical geneticist (J.B.). The first patient had a paternally inherited triplication on the X chromosome. The second patient carried two additional CNVs, in particular a benign 7q26.3 deletion and a clinically irrelevant 9q31 duplication. The third patient had a small microduplication on the Y chromosome. The fourth patient had a small 15q11.2 deletion with presumed minor effect on the phenotype. The majority of patients (72%, 23/32) carry the most common 3 Mb duplication, located at LCR22A–LCR22D. One patient had breakpoints situated at LCR22A–LCR22B, one at LCR22A–LCR22C, one at LCR22A–LCR22F, and two patients at LCR22C–LCR22D (Supplementary Table 2.1). The four remaining patients (4/32) carry a distal duplication: two siblings had a distal LCR22E–LCR22F duplication, one patient with the proximal breakpoint located proximal to LCR22F and one patient has unique breakpoints distal to LCR22D. The four patients with distal duplications were excluded due to the lack of gene overlap in the duplication region and its potential impact on the clinical phenotype.

For the current study, 28 patients with 22q11.2Dup between 4.1 and 56.7 years (median age = 17.11 years) were included, 11 females and 17 males (see Table 2.1). The average gestational age was 38.8 weeks with a wide range from 1 patient born at 30 weeks of gestation to a quarter of patients (5/20) born at 41 weeks of gestation. Using a genetic first approach, all patients were diagnosed with the 22q11.2Dup after investigation by microarray analysis, except for three patients who had originally received the diagnosis by FISH

investigation. These three patients underwent an additional microarray to delineate the size of the duplication. The used platforms to diagnose the duplication were 1-Mb resolution Bacterial Artificial Chromosome (BAC) array combined with a full tiling array of Chromosome 22, OGT 180k CytoSure ISCA v2, 180k CytoSure constitutional v3, Illumina SNP12V2, OGT 105K array, and SNP array 6.0 (Supplementary Table 2.1).

Table 2.1 – Demographic data, genetic data, and clinical features in 28 patients with proximal 22q11.2Dup.

	Duplication 22q11.2 (*n* = 28) *n* (%)	
Demographics	Sex	Male: 17/28 (61%)
		Female: 11/28 (39%)
Genetic data	Breakpoints	A-D 23/28 (82%)
		C-D 2/28 (7%)
		A-B 1/28 (3%)
		A-C 1/28 (4%)
		A-F 1/28 (4%)
	Inheritance	Inherited: 13/21 (62%)
		- maternal: 7/13 (54%)
		- paternal: 6/13 (46%)
		De novo: 8/21 (38%)
Medical phenotype and clinical features	Dysmorphism *	18/28 (64%)
	Congenital heart defect	8/24 (33%)
	Nutritional problems	12/21 (57%)
	Failure to thrive	7/21 (33%)
	Short stature	5/24 (21%)
	Abnormal head size	9/28 (32%)
	Genitourinary problems	5/21 (24%)
	Otorhinolaryngology (Ear Nose Throat)	
	Palatal defects	6/23 (26%)
	Hearing impairment	12/23 (52%)
	Neurological abnormalities	
	Epilepsy	3/26 (12%)
	Hypotonia	7/26 (27%)
Dysmorphic features (18/28 – 64%)	Unilateral cleft lip/palate	2/28 (7%)
	Thin upper lip	4/28 (14%)
	Smooth philtrum	3/28 (11%)

	Up/downslanting palpebral fissures	5/28 (18%)
	Hypo/hypertelorism	5/28 (18%)
	Epicanthic folds	4/28 (14%)
	Strabism	3/28 (11%)
	Minor ear anomalies	7/28 (25%)
	Minor nose anomalies	4/28 (14%)
	Minor hand/feet anomalies	5/28 (18%)
	Facial dysmorphic features	15/28 (54%)

Note. *for more details on dysmorphism see under dysmorphic features in Table 2.1.

Twenty-six patients are index patients, while two patients are siblings of the index patient. This is the case for Patient 5 and 6 (two sisters) and Patient 13 and 14 (brother and sister). Both index patients and siblings were identified through molecular karyotyping in a diagnostic setting due to developmental or medical issues, whereas other carrier family members mentioned in the study were only diagnosed because of segregation analysis and not based on their clinical presentation. The 22q11.2Dup was inherited from a parent in 62% of the patients (13/21 cases), while 38% (8/21) occurred *de novo*. In the remaining patients ($n = 7$), no information was available on the inheritance pattern.

2.2.3 Chart review

We conducted a retrospective analysis of the digital medical records of all 28 patients with a 22q11.2Dup, in whom data have been collected for several reasons, including diagnostics and clinical follow-up. As a consequence, a variable amount of data is available across different domains and patients. Therefore, the total number of patients can differ dependent on the available data in the described domain. A subgroup of 10 patients has previously been published by Van Campenhout et al., 2012. The digital medical records included data on genetics, medical history, clinical features, developmental milestones, and behavioural characteristics. Data on developmental milestones, indicating whether milestones in different developmental domains were reached in time, were based on clinical follow-up or developmental anamnesis provided by parents. Developmental and behavioural difficulties refer to manifestations that were described in medical records by healthcare professionals and were assessed with screening instruments and questionnaires such as Child Behavior Checklist (CBCL) and Teacher's Report Form (TRF, Achenbach & Rescorla, 2001). Only parent and teacher reports were included. All diagnoses of NDDs and psychiatric disorders were made by a multidisciplinary team, consisting of healthcare

professionals, child and adolescent psychiatrists, or paediatric neurologists, using standardised diagnostic instruments such as Autism Diagnostic Observation Schedule – Second Edition (ADOS-2) and Autism Diagnostic Interview – Revised (ADI-R) for ASD, and psychiatric interviews, according to Diagnostic and Statistical Manual of Mental Disorders, fifth version (DSM-5) criteria (American Psychiatric Association, 2013; de Bildt et al., 2013; de Jonge et al., 2003; Lord et al., 2012; Rutter et al., 2003). More details on the administered screening and diagnostic instruments can be found in Supplementary Table 2.2 online.

Depending on the age of the participants, the following standardised intelligence tests were administered in 19 patients: the Dutch editions of Bayley Scales of Infant Development – Second edition (BSID-II-NL), Wechsler Preschool and Primary Scale of Intelligence Revised (WPPSI-R), Snijders – Oomen Nonverbal test Revised (SON-R), Wechsler Intelligence Scale for Children – Third Edition (WISC-III-NL), and Wechsler Intelligence Scale for Children – Fifth Edition (WISC-V-NL) (Bayley, 1993; Kort et al., 2005; Tellegen et al., 1998; van der Meulen et al., 2004; Vander Steene & Bos, 1997; Wechsler, 1989, 1991, 2002, 2014, 2018; Wechsler et al., 2009). A small subgroup underwent formal cognitive assessment at two or three time points. In the youngest group, first assessment was done by means of BSID-II-NL, while WPPSI-III or SON-R was used for the second assessment (Bayley, 1993; Tellegen et al., 1998; Wechsler, 1989). In the oldest group, IQ assessment was done by means of WPPSI, SON-R, or WISC at the two timepoints (Tellegen et al., 1998; Wechsler, 1991, 2002, 2014).

Regarding longitudinal data on language, six patients underwent standardised language assessment on at least two different occasions. The following standardised language tests were administered, depending on the age of the patient at time of testing: the Dutch versions of the Reynell Developmental Language Scales, Schlichting Test for Language Production, and Clinical Evaluation of Language Fundamentals (Kort et al., 2010; Schaerlaekens et al., 2003; Schlichting & Lutje Spelberg, 2010; Semel et al., 2010).

2.2.4 Statistical analysis

Proportional differences in the clinical and developmental phenotype of *de novo* and inherited duplications were compared using Fisher's exact tests by means of JASP (JASP Team, 2020). Bonferroni correction was applied for

multiple testing resulting in a significance level of 0.05/28 = 0.0018. Cramer's V was executed as effect size parameter with values from 0.10 to 0.30 demonstrating small effect size, values from 0.30 to 0.50 expressing a medium effect size and values 0.50 illustrating a large effect.

2.3 Results

2.3.1 Age of diagnosis and presenting symptoms

Patients received the diagnosis of 22q11.2Dup at a median age of 8 years, with a wide range from one prenatal diagnosis to one at the age of 51. Sixty-one percent (17/28) of patients were diagnosed before the age of 10, whereas 29% (8/28) received a diagnosis between 10–20 years. Reasons for diagnosis were medical features in 36% (10/28), developmental difficulties in 39% (11/28) and both developmental and medical concerns in 25% (7/28). The first category includes congenital heart defects, short stature, epilepsy, dysmorphism, feeding problems, failure to thrive, cleft lip/palate, hyperlaxity of joints, and skeletal dysplasia. Developmental concerns refer to developmental delay in 54% (15/28) of patients, ASD in 7% (2/28) and motor and speech-language delay in one patient.

2.3.2 Clinical features

In general, 64% (18/28) of patients show mild and variable dysmorphic features. Patients demonstrate heterogeneous phenotypic representations with variable major congenital anomalies. Common medical issues, listed in descending order of occurrence, include: nutritional problems, transient or permanent hearing impairment, congenital heart defects, failure to thrive, abnormal head size, muscular hypotonia, palatal defects, genitourinary abnormalities and short stature (see Table 2.1). More than half (12/21) of patients have a history of nutritional problems, referring to feeding difficulties during the first months including difficulties with swallowing, sucking, allergies and frequent vomiting. Three of them experienced gastroesophageal reflux in the past, of whom two suffered from severe nutritional problems requiring tube feeding. Nutritional problems might cause failure to thrive, which is described in 33% (7/21) of patients. However, two patients with failure to thrive did not experience nutritional problems.

In more than half (12/23) of patients transient or permanent hearing problems are seen. Recurrent ear infections occurred in 35% (8/23) of patients, requiring tympanostomy tubes, whereas permanent hearing impairment is

observed in 9% (2/23) of patients, comprising one with mild hearing impairment and one with conductive hearing loss, affecting language development. Palatal defects are found in approximately a quarter (6/23) of patients and involve unilateral cleft lip/palate (n = 2), high palate (n = 2), and bifid uvula (n = 2). Structural congenital heart defects are present in one-third (8/24) and comprise arteria lusoria, atrioventricular septum defect, coronary heart disease, transposition of the great arteries, aortic coarctation and hypoplastic arch, atrial septum defect Type 2, and patent ductus arteriosus. Neurological abnormalities are noted in 39% (10/26) including muscular hypotonia in 27% (7/26) and epilepsy in 12% (3/26). In addition, 12% (3/26) of patients had aspecific brain anomalies (Magnetic Resonance Imaging, MRI) including periventricular leuco-encephalomalacia, mild asymmetry of lateral ventricles in combination with gait abnormalities and parietal and frontal periventricular white matter lesions.

Twenty-one percent (5/24) have growth delay or short stature, whereas microcephaly or macrocephaly were reported in 5/28 (18%) and 4/28 (14%), respectively. Other skeletal anomalies include hyperflexible joints, kyphosis and pes planus, mild scoliosis, hip dysplasia, and rhizomelic and mesomelic shortening of the limbs, limited elbow extension and limited hip abduction. Finally, in almost a quarter of patients (5/21) genitourinary problems are identified, involving two males with cryptorchidism.

2.3.3 Developmental and behavioural phenotype

Patients with the 22q11.2Dup are characterised by a notably variable developmental and behavioural phenotype. In Table 2.2, information is provided on the developmental history of patients, indicating the emergence of speech and language milestones was delayed in 68% (17/25), whereas delay of motor milestones occurred in 58% (15/26). Moreover, the majority (73%, 19/26) of patients demonstrate developmental delay, ranging from borderline intelligence to moderate intellectual disability (ID) (Table 2.2).

Cognitive development was assessed by means of standardised IQ tests in 19 patients. Ten percent (2/19) of patients have an IQ below 55 (moderate ID), 21% (4/19) have an IQ in the mild ID range of 55–70, 37% (7/19) have a borderline IQ (IQ 71–85), and approximately one-third (6/19) have an IQ within the normal range (IQ 86–115). A median value of 76 (range IQ 51–114) is found for full-scale IQ (FSIQ), whereas performance (PIQ) and verbal IQ (VIQ) have a median value of 87 (range IQ 53–115) and 84 (range IQ 54–120), respectively.

A disharmonic profile, defined as a VIQ–PIQ discrepancy of at least 15 IQ points based on the Dutch administration manual of the WISC-III, is noted in 42% (6/14) of patients (Graauwmans et al., 2017): in 5 patients PIQ exceeds VIQ, while only in 1 patient VIQ is significantly higher than PIQ.

The category of developmental and behavioural difficulties in Table 2.2 refers to manifestations that are described in medical records by healthcare professionals, based on screening instruments, whereas NDDs and psychiatric disorders are diagnosed based on standardised diagnostic instruments. The most common reported developmental and behavioural difficulties are attention problems in 64% (16/25) of patients. While only one patient was diagnosed with attention deficit disorder (ADD), 10 patients received the diagnosis of ADHD, of whom 8 have been treated with medication (methylphenidate). Learning problems were present in 60% (15/25), of whom four have a specific learning disorder (SLD): three with severe reading difficulties and one with a combined reading and writing difficulty.

Persisting motor problems occurred in 13 patients, despite the fact that 3 of them had no history of motor delay. Motor problems consist of gross motor problems in 15% (2/13), fine motor problems in 38% (5/13) and combined fine-gross motor impairment in 46% (6/13) of patients. In addition, three patients with motor problems were diagnosed with developmental coordination disorder (DCD). On the domain of visual perception skills and visuomotor integration, half (9/18) of patients experience problems, of whom 3 patients only had visual-motor problems, while 6 had combined visual-motor and visual perception problems. Two of them were eventually diagnosed with cerebral visual impairment (CVI).

Behavioural problems occur in 40% (10/25) of patients and have been ascertained in 8 patients based on parental or teacher questionnaires. One patient experiences impulsiveness, aggressive outbursts and has a reactive attachment disorder. A second person suffers from severe behavioural difficulties with aggressive outbursts and was eventually diagnosed with oppositional defiant disorder, which has been treated with risperidone. Twenty-nine percent (7/24) of patients present with autistic traits, including being socially immature, shy, facing anxieties, or scoring in the clinical range without having a formal diagnosis of ASD. Three of them were actually diagnosed with ASD. As for the patients without autistic traits, one patient was too young to assess and another one was on the waiting list for ASD diagnostic assessment. In one person language

difficulties persisted and resulted in a developmental language disorder. Further psychiatric comorbidity was observed: 20% (5/25) of patients suffer from anxiety, 1 person had depressive symptoms, for whom an admission to hospital was required, and the oldest patient suffers from schizophrenia.

Table 2.2 – Developmental and behavioural characteristics and psychiatric diagnoses in proximal 22q11.2Dup (*n* = 28).
Manifestations described in medical records by healthcare professionals.

Duplication 22q11.2 (*n* = 28)		
Intellectual functioning	IQ range	<55: 2/19 (10%) 55-70: 4/19 (21%) 71-85: 7/19 (37%) 86-100: 3/19 (16%) 101-115: 3/19 (16%)
Developmental history - Milestones	Developmental delay	19/26 (73%)
	Speech-language delay	17/25 (68%)
	Motor delay	15/26 (58%)
Developmental - Behavioural difficulties*	Attention problems	16/25 (64%)
	Autistic traits	7/24 (29%)
	Learning problems	15/25 (60%)
	Behavioural problems	10/25 (40%)
	Anxiety	5/25 (20%)
	Motor problems	13/25 (52%)
	Visual perceptual/motor problems	9/18 (50%)
	Speech-language problems	4/8 (50%)
Neurodevelopmental and psychiatric disorders**	Attention-Deficit/Hyperactivity disorder	11/25 (44%)
	Autism Spectrum Disorder	3/24 (13%)
	Specific learning disorder	4/25 (16%)
	Developmental coordination disorder	3/25 (12%)
	Cerebral visual impairment	2/18 (11%)
	Developmental language disorder	1/8 (13%)
Education - Therapy	Special education	12/23 (52%)
	Therapy	18/21 (86%)

Note. * assessed with screening instruments and questionnaires such as CBCL, TRF (Achenbach & Rescorla, 2001).

** assessed with standardised diagnostic instruments such as ADOS, ADI-R (Lord et al., 2012; Rutter et al., 2003), psychiatric interviews, according to DSM-5 criteria (American Psychiatric Association, 2013).

2.3.4 Longitudinal developmental and behavioural data

A small subgroup of patients underwent formal cognitive assessment at two or three time points. In the youngest subgroup, the first test was administered in early toddlerhood (median age of 1.10 years, range 1.7–2.5 years), while the next assessment was done in preschool (median age of 4.7 years, range 3.3–5.2 years). The average IQ difference between these 2 time points was 15 favouring the second measurement. In Figure 2.2, IQ data of the oldest subgroup are visualised: The first IQ assessment took place at a median age of 5.2 years (range 3.8–9 years), whereas the second assessment was conducted at a median age of 8.8 years (range 5.10–16.4 years).

FSIQ data (n = 11) in Figure 2.2 illustrate that almost two-third of patients (64%, 7/11) have a relative stable cognitive trajectory, while approximately one-third of patients (36%, 4/11) show a growing into deficit trajectory, displayed by the gap of more than 10 IQ points between the two time points. A relative stable trajectory is characterised by increasing raw scores on subtests indicating adequate progress, while scaled and standard scores remain stable over time. Growing into deficit or developmental lag refers to patients who are making insufficient progress with increasing age, resulting in a growing discrepancy in relation to their typically developing peers. They develop at a slower pace compared to the general population causing decreased scaled and standard scores on certain subtests (Swillen & McDonald-Mcginn, 2015; Van Den Heuvel et al., 2018). As shown in Figure 2.2, PIQ and VIQ trajectories are comparable to those of FSIQ, although patient 11 obtains remarkable higher PIQ than FSIQ scores at the second time point, which can be regarded as a catch up on the performance domain. The complete intellectual profile with FSIQ, PIQ, and VIQ is only available for four patients because sometimes only FSIQ data were mentioned in patient records or only a nonverbal IQ test such as SON-IQ was administered. Regarding language, longitudinal data are available for six patients, with median age of 3.5 years (range 2.7–6.3 years) at first assessment and 5.5 years (range 3.4–8.8 years) at second assessment. These results suggest that three of them made sufficient progress resulting in a relative stable trajectory, while one patient caught up with peers and two patients have grown into deficit.

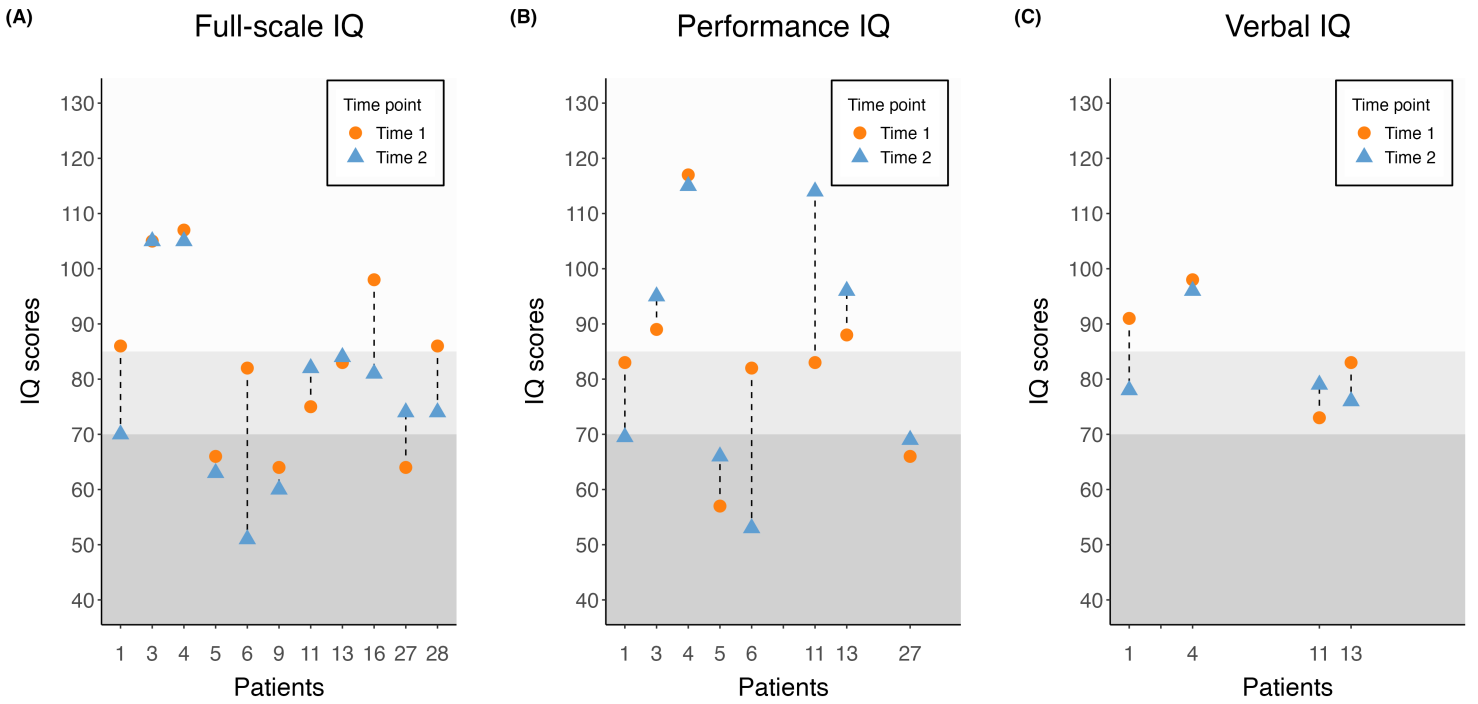


Figure 2.2 – Longitudinal IQ information: IQ scores per patient at two different timepoints.
A) Full-scale IQ of 11 patients, B) Performance IQ of eight patients, C) Verbal IQ of four patients

Data on education were available for 23 patients: 7 out of 23 patients followed or have been following regular education without support, and 4 patients with additional educational support. Twelve patients have attended special education (12/23, 52%), either from the start (8/12) or transitioning from regular education to special education (4/12). Six of these patients followed special education for children with mild ID or special educational needs for whom the regular program is not achievable, four patients attend special education for children with moderate to severe ID and the remaining two follow special education for children with ASD. Regarding therapy, the available information on 21 patients indicates that almost all patients (18/21) received therapy during early infancy. The majority (70%, 14/20) received speech-language therapy for a speech-language delay, articulation problems, language difficulties, and specific learning disorders like dyslexia. Sixty-three percent (12/19) received physiotherapy to improve gross or fine motor skills. Home-based early intervention was organised in 5 out of 17 families (29%). Three children (16%) received occupational therapy, and two children play therapy.

2.3.5 Phenotypic differences between *de novo* and inherited duplications

The duplication was inherited in 13 cases (13/21; 62%), of which 46% (6/13) were paternal and 54% (7/13) maternal, occurred *de novo* in 8 cases (8/21; 38%) and the inheritance pattern was unknown in 7 patients (7/28), of whom 4 had a positive family history of developmental delay. Looking at the familial duplications (n = 13), only three patients had parents without phenotypic features, while in the remaining families parents or siblings showed phenotypic features in variable degrees: most reported features include developmental delay, family members requiring special education, and diagnoses of NDDs.

A comparison between the phenotype of *de novo* and familial duplications reveals no significant differences, although several trends are observed (see Table 2.3). Almost all patients (7/8) with *de novo* duplications show dysmorphic features, while only 54% (7/13) of patients with familial duplications present with dysmorphic features (p = 0.174). More patients with *de novo* duplications demonstrate congenital heart defects (p = 0.332), failure to thrive (p = 0.013), short stature (p = 0.245), palatal defects (p = 0.129), and hypotonia (p = 0.305) compared to patients with familial duplications. In contrast, epilepsy has only been reported in patients with familial duplications (p = 0.509). Within the developmental and behavioural domain, the occurrence of delayed milestones is similar in both duplication groups, except for speech-language delay being more

noticed in patients with familial duplications (p = 0.356). More patients with *de novo* duplications suffer from anxiety (p = 0.347), while more patients with familial duplications experience learning problems (p = 0.356). In addition, DCD (p = 0.495) and SLD (p = 0.242) were only diagnosed in patients with familial duplications. Remarkably, only a quarter (2/8) of patients with *de novo* duplications attend special education, while 63% (7/11) of patients with familial duplications follow special education (p = 0.170). Finally, patients with *de novo* duplications have on average a higher FSIQ value compared to patients with inherited duplications (86 vs. 79, p = 0.494), with similar values for PIQ (87 vs. 89, p = 0.836), but higher values for VIQ (90 vs. 80, p = 0.347).

Cramer's V indicates a large effect size for proportional differences in failure to thrive (0.645), while medium effect sizes can be observed for differences in dysmorphic features (0.347), short stature (0.396), palatal defects (0.420), SLD (0.343), and special education (0.382). Comparing the phenotypes of maternal (n = 7) and paternal (n = 6) inherited duplications, no significant differences are found. However, more patients with paternal duplications tend to have developmental delay (p = 0.545, Cramer's V = 0.354), attention problems (p = 0.061, Cramer's V = 0.707), autistic traits (p = 0.182, Cramer's V = 0.559), motor problems (p = 0.242, Cramer's V = 0.507), and visual perceptual problems (p = 0.524, Cramer's V = 0.408).

Table 2.3 – Phenotypic differences between *de novo* (n = 8) and inherited (n = 13) duplications

		***De novo* (8)** ***n* (%)**	**Inherited (13)** ***n* (%)**	**Fisher's exact**	**Cramers' *V***
Medical phenotype and clinical features	Dysmorphism	7/8 (88%)	7/13 (54%)	p = 0.174	0.347*
	Congenital heart defect	4/7 (57%)	3/11 (27%)	p = 0.332	0.299
	Nutritional problems	4/7 (57%)	6/11 (55%)	p = 1	0.025
	Failure to thrive	5/7 (71%)	1/11 (9%)	p = 0.013	0.645**
	Short stature	3/7 (43%)	1/11 (9%)	p = 0.245	0.396*
	Abnormal head size	3/8 (38%)	6/13 (46%)	p = 1	0.085
	Genitourinary problems	2/7 (29%)	2/11 (18%)	p = 1	0.122
	ENT: Palatal defects	4/7 (57%)	2/12 (17%)	p = 0.129	0.420*
	ENT: Functional ear problems	4/7 (57%)	6/12 (50%)	p = 1	0.069
	Neurology: Epilepsy	0/7 (0%)	2/12 (17%)	p = 0.509	0.262
	Neurology: Hypotonia	3/7 (43%)	2/12 (17%)	p = 0.305	0.287
Developmental history - Milestones	Developmental delay	6/8 (75%)	8/12 (67%)	p = 1	0.089
	Speech-language delay	4/8 (50%)	9/12 (75%)	p = 0.356	0.257
	Motor delay	5/8 (63%)	8/12 (67%)	p = 1	0.043
Developmental - Behavioural difficulties	Attention problems	5/8 (63%)	8/12 (67%)	p = 1	0.043
	Autistic traits	4/8 (50%)	3/11 (27%)	p = 0.377	0.233
	Learning problems	4/8 (50%)	9/12 (75%)	p = 0.356	0.257
	Behavioural problems	3/8 (38%)	5/12 (42%)	p = 1	0.042
	Anxiety	3/8 (38%)	2/12 (17%)	p = 0.347	0.236

	Motor problems	4/8 (50%)	7/12 (58%)	$p = 1$	0.082
	Visual perceptual/motor problems	3/6 (50%)	5/10 (50%)	$p = 1$	0.000
Neurodevelopmental and psychiatric disorders	Attention-Deficit/Hyperactivity disorder	3/8 (38%)	6/12 (50%)	$p = 0.670$	0.123
	Autism Spectrum Disorder	2/8 (25%)	1/11 (9%)	$p = 0.546$	0.215
	Specific learning disorder	0/8 (0%)	3/12 (25%)	$p = 0.242$	0.343*
	Developmental coordination disorder	0/8 (0%)	2/12 (17%)	$p = 0.495$	0.272
	Cerebral visual impairment	1/5 (20%)	1/10 (10%)	$p = 1$	0.043
Education – Therapy	Special education	2/8 (25%)	7/11 (64%)	$p = 0.170$	0.382*
	Therapy	6/7 (86%)	9/11 (82%)	$p = 1$	0.051

Note. * medium effect; ** large effect
Abbreviations: ENT, ear nose throat

2.4 Discussion

This systematic chart review reports on the clinical and developmental phenotype of 28 patients harbouring the proximal 22q11.2Dup, and on longitudinal data in a small subgroup. The most reported indications for diagnosis were developmental difficulties (39%), medical issues (36%), and combined medical and behavioural difficulties (25%). The prevalence of medical and combined medical-behavioural indications is in agreement with the findings of Wenger et al. (2016), although, in this chart review prevalences for developmental concerns were higher and familial history was not reported as an indication for diagnosis. Median age at genetic diagnosis was 8 years opposed to an average of 4.5 years (Wenger et al., 2016). Congenital defects with immediate or major functional impact such as major congenital heart defects were typically discovered early in life, whereas patients with epilepsy or feeding problems were usually diagnosed at a later stage. The high proportion of typical A–D duplications in this cohort is in agreement with other studies, as well as the proportion of inherited duplications (Clements et al., 2017; Dupont et al., 2015; Woodward et al., 2019).

The clinical phenotype in proximal duplications reveals a wide heterogeneous phenotype including dysmorphic features (64%), nutritional problems (57%), transient or permanent hearing impairment (52%), failure to thrive (33%), congenital heart defects (33%), abnormal head size (32%), neurological abnormalities such as hypotonia (27%) and epilepsy (12%), genitourinary problems (24%), short stature (21%), and variable involvement of other organs and systems, which is in line with the literature (Butensky et al., 2020; Clements et al., 2017; Dupont et al., 2015; Ensenauer et al., 2003; Firth, 2013; Portnoï, 2009; Van Campenhout et al., 2012; Wenger et al., 2016; Woodward et al., 2019; Yobb et al., 2005; Zhang et al., 2021). Regarding congenital heart defects, most studies reported a similar number of prevalence (13%–27%) (Butensky et al., 2020; Clements et al., 2017; Dupont et al., 2015; Ensenauer et al., 2003; Portnoï, 2009; Van Campenhout et al., 2012; Wenger et al., 2016; Zhang et al., 2021), except for two studies finding 0% and 83% of patients with heart defects (Woodward et al., 2019; Yu et al., 2019). Differences in reported prevalence can often be explained by ascertainment bias in different research settings, such as university hospitals compared to psychiatric hospitals, or by small sample sizes. Hence, multicentre studies with diverse populations and large sample sizes are required to determine the true prevalence of congenital heart defects in patients with 22q11.2Dup.

In the majority of patients with duplications, the developmental-behavioural phenotype is characterised by developmental delay, impacting different developmental domains with variable degrees of severity. Developmental (73%), speech-language (68%), and motor (58%) delay are common in infancy, while attention (64%), learning (60%), motor (52%), visual perceptual (50%), and behavioural problems (40%) are typically reported at primary school age. Most common NDDs are AD(H)D (44%), SLD (16%), and ASD (13%), which is – for AD(H)D and ASD – in line with the literature, however, diagnoses of SLD, DCD, and CVI have not been reported yet in previous studies (Chawner et al., 2019; Lin et al., 2020; Olsen et al., 2018; Ou et al., 2008; Portnoï, 2009; Van Campenhout et al., 2012; Wenger et al., 2016; Woodward et al., 2019; Yu et al., 2019; Zhang et al., 2021).

Regarding cognitive functioning, we found a median FSIQ level in the borderline range (IQ 76), with one-third of patients functioning in the borderline range (FSIQ 71–84) and one-fifth of patients suffering from a mild ID (FSIQ 55–70), which is lower compared to the findings of Chawner et al. (2021). In addition, the present study provides for the first time longitudinal developmental, cognitive, and behavioural data on a small subgroup of patients. In the youngest group, with first assessment in toddlerhood (age 1.11 years) and second assessment at preschool age (age 4.4 years), the average difference in IQ is 15 IQ points favouring the second measurement, which might indicate that the capacities of these children were underestimated by BSID-II-NL or overestimated by WPPSI-III and SON-R. Another possible explanation is that in infancy their overall development and functioning were dominated by major congenital anomalies such as congenital heart defects or hypotonia. In the older group (primary school age to adolescence), two-third of patients (7/11) showed a relative stable cognitive trajectory, whereas one-third showed (4/11) a growing into deficit trajectory with increasing age. Regarding language, two-third (4/6) made sufficient progress or caught up with peers, while one-third (2/6) showed a growing into deficit profile, which is predominantly in agreement with their cognitive trajectories. This increasing cognitive deficit with age needs to be interpreted with caution, since it might be partially caused by measurement confound or age range differences. However, growing into deficit is often observed in children with CNVs (Swillen & McDonald-Mcginn, 2015; Van Den Heuvel et al., 2018) and may be partially explained by the increasing proportion of abstract reasoning skills in IQ tests when children grow older. This finding is in line with the observation that an increasing number of 22q11.2Dup patients need additional educational support or even change from regular to special

education with increasing age. In early infancy, almost all patients require therapy due to delayed motor and speech-language milestones. When entering primary school, children are confronted with increasing learning and social challenges in school, which sometimes resulted in more frustrations and behavioural problems. Consequently, additional support with more diverse and specialised educational interventions is needed.

Regarding further psychiatric comorbidity, current literature provides contradictory findings on the presence of schizophrenia in patients with 22q11.2Dup: Rees et al. (2014) failed to find patients with 22q11.2Dup in a schizophrenia cohort and concluded that the duplication has a protective factor against schizophrenia, whereas Van Amelsvoort et al. (2016) stated that this conclusion was too premature by reporting on a case with 22q11.2Dup and a comorbid psychotic disorder fulfilling the criteria of schizophrenia. In the current study, only one patient suffered from schizophrenia, which might be explained by the fact that a rather young cohort (median age of 17.11 years with only one patient older than 35 years) was described. Therefore, longitudinal follow-up of these patients into adulthood is needed to determine whether a true association exists between 22q11.2Dup and schizophrenia or whether the case reported in the current study is a coincidental finding.

Given these findings, the current cohort of patients with proximal 22q11.2Dup seems to be representative for what has been described in the literature so far. Both interfamilial heterogeneity and intrafamilial heterogeneity are noticed among patients with 22q11.2Dup. Some parents with 22q11.2Dup had a mild to near-normal phenotype and were only diagnosed because the duplication was found in their affected child. Therefore, a number of individuals with 22q11.2Dup with a near-normal phenotype presumably remain unnoticed, making it hard to predict the true prevalence of this CNV in the general population, and hence the penetrance of medical or developmental issues related to 22q11.2Dup (Firth, 2013; Portnoï, 2009). The variable expressivity of the 22q11.2Dup may be caused by different factors. One potential explanation is the presence of additional genetic variants. Therefore, we excluded patients carrying known additional CNVs with clinical impact a priori. Nevertheless, patients in the current study did not undergo additional genetic testing, but whole-genome sequencing or trio whole-exome sequencing should be performed in all patients and their families to detect additional *de novo* or inherited genetic variants such as single-nucleotide variants or CNVs, with potential clinical impact. Another possible explanation of variability can be found in the size and location of the

duplication. Although previous studies suggest that the size of the duplication is not a reliable prediction for phenotypic expressivity, larger studies with atypical and distal duplications are needed to compare the 3 Mb duplication to smaller or larger overlapping 22q11.2Dups (Dupont et al., 2015; Ensenauer et al., 2003; Portnoï, 2009).

Although we found no statistically significant differences between patients with *de novo* versus inherited duplications and paternal versus maternal duplications, we observed some trends in phenotypic differences. Except for epilepsy, patients with *de novo* duplications in this sample present with more major congenital anomalies like congenital heart defects, failure to thrive, short stature, palatal defects, and hypotonia. In contrast, the majority of patients with inherited duplications demonstrate speech-language delay and attend special education. Moreover, patients with inherited duplications suffer from more NDDs such as learning disorders and DCD. However, a positive family history may contribute to these developmental problems and NDDs in CNVs, acting as an additional genetic burden and making patients susceptible to more severe phenotypes. Pizzo et al. (2019) found that in 16p12.1 deletion and duplication the severity and variability of the developmental and behavioural phenotype is dependent on the family history of NDDs. In particular, persons with CNVs and a strong family history showed more severe clinical features opposed to those without or with a mild family history (Pizzo et al., 2019). In addition, other environmental and social factors may play a role in these families opposed to families with *de novo* events. One of these social factors found in patients with familial 22q11.2DS is the lower educational attainment level of both parents, caused by the CNV in the affected parent and influenced by the principle of assortative mating resulting in a lower educational level in the unaffected parent (De Smedt et al., 2007). Therefore, it is important to take into account both the broader genetic and environmental contexts.

In this study, we confirm that patients with 22q11.2Dup present higher cognitive functioning and fewer major congenital anomalies, compared to the patient population with 22q11.2DS (Lin et al., 2020; Olsen et al., 2018; Wenger et al., 2016). Individuals with 22q11.2Dup show a similar spectrum of birth defects, medical, and behavioural problems as patients with 22q11.2DS, but at lower rates (Goldenberg, 2018). Another finding is that patients with 22q11.2DS are often diagnosed with the inattentive ADD type without hyperactivity, while patients with 22q11.2Dup receive more commonly the combined diagnosis of ADHD (Niarchou et al., 2015; Olsen et al., 2018; Ousley et al., 2007). In addition,

deletions are usually discovered at a younger age due to the more urgent and severe medical issues apparent at an earlier stage in life, whereas duplications more often came to attention with developmental and behavioural issues. This milder phenotypic expression found in patients with duplications compared to deletions also applies to several other CNVs, such as 7q11.23 deletion and duplication (Goldenberg, 2018).

2.4.1 Limitations, strengths, and future

The genetic-first approach in this chart review may introduce bias in the described phenotype and hence not cover the whole spectrum of presentations in the patients with the 22q11.2Dup, because mainly patients with discernible phenotypes have been discovered so far. Only index patients with medical or cognitive problems were included, which might result in an ascertainment bias. To delineate the phenotype of 22q11.2Dup by means of unbiased methods, future studies should only include family members with 22q11.2Dup diagnosed through segregation analysis and exclude index patients. The inclusion of two siblings of index patients in the current study might also introduce bias since they share the same environmental factors such as parental educational attainment and socioeconomic status, which might impact the phenotype (De Smedt et al., 2007).

As the current chart review reports on data from a relatively small sample, our ability to make generalised statements about the whole 22q11.2Dup population is limited. In addition, the limited number of patients with *de novo* versus familial duplications restricts our ability to draw general conclusions about the clinical impact of these inheritance patterns. However, large-scaled studies on the phenotype of the 22q11.2Dup are scarce and a substantial part of the current literature is on case report-level. Therefore, the given results are still of high interest for medical healthcare professionals. Future large-scaled and multicentre prospective studies using a standardised common protocol such as the IBBC-rareCNV consortium are needed to get more insight in the developmental and behavioural phenotype of the 22q11.2Dup and to confirm the observed differences in phenotype between *de novo* and inherited duplications.

A key strength of the current study is that all data come from digital medical records, including medical reports and standardised tests administered by healthcare professionals. However, because of the retrospective nature of the study, available data were limited, particularly in the case of older patients, and the data were collected without the use of a systematic standardised protocol of

tests, which could result in certain methodological shortcomings. Finally, this study provides for the first time longitudinal IQ data in a small subgroup of patients with 22q11.2Dup. More extensive prospective longitudinal studies are required to elucidate the cognitive trajectories in patients with the 22q11.2Dup throughout the lifespan.

2.5 Conclusions

This study provides physical, developmental, and behavioural data on index patients with proximal 22q11.2Dup, provides longitudinal IQ data in a small subgroup, and reports for the first time on trends of phenotypic differences between patients with *de novo* and inherited duplications in this region. These findings are relevant to medical healthcare professionals, such as paediatricians, child and adolescent psychiatrists, and professionals working at different care settings such as special education, rehabilitations centres, and hospitals, and may help to guide medical and neurobehavioural follow-up. When children are diagnosed with 22q11.2Dup prenatally or early in life, healthcare professionals should be aware of an increased risk of nutritional problems, heart defects, and hearing problems, and should initiate neurodevelopmental support early in life, given the high prevalence of developmental delay, learning, or behavioural problems.

2.6 Supplementary material

Supplementary Table 2.1 – Additional genetic information on the 28 patients with 22q11.2Dup in this chart review. Chromosomal position according to Hg19, region, size and break points of the duplications.

Patient number	Region	Chromosomal positions - Hg19	Size (Mb)	Test used - more specific	Break Points
1	22q11.21q11.23	18,844,632-24,977,286	6.1	Array: Illumina SNP12V2.1	A-F
2	22q11.21	18,861,748-21,462,353	2.6	Array: Illumina SNP12V2	A-D
3	22q11.21	18,628,147-22,123,338	3.49	FISH for 22q11 deletion - delineated by array CGH: 180k Cytosure ISCA v2 (OGT)	A-D
4	22q11.21	18,643,474-21,759,580	3.1	FISH for 22q11 deletion - delineated by array CGH: 180k Cytosure ISCA v2 (OGT)	A-D
5	22q11.21	18,468,000-21,926,000	3.4	Array: 1 Mb resolution BAC array > delineated by full tiling array of chromosome 22	A-D
6	22q11.21	18,468,000-21,926,000	3.4	Array: 1 Mb resolution BAC array > delineated by full tiling array of chromosome 22	A-D
7	22q11.21q11.22	21,798,813-22,957,026	1.1	Array: 105K OGT array	C-D
8	22q11.21	18,890,162-20,312,008	1.4	Array: OGT 180k Cytosure ISCA v2	A-B
9	22q11.21	18,585,000-18,983,507>21,944,643--22,670,000	3-4.1	Array: 1 Mb resolution BAC array	A-D

10	22q11.21	18,890,419-21,462,447	2.5	Array: Affymetrix SNP Array 6.0	A-D
11	22q11.21	19,204,025-21,244,003	1	Array: 105K OGT array	A-C
12	22q11.21	18,765,102-21,441,944	2.6	FISH for 22q11 deletion - delineated by array CGH: 180k Cytosure ISCA v2 (OGT)	A-D
13	22q11.21	18,875,830-21,441,944	2.6	Array: OGT 180k Cytosure ISCA v2	A-D
14	22q11.21	18,875,830-21,441,944	2.6	Array: OGT 180k Cytosure ISCA v2	A-D
15	22q11.21	18,875,830-21,883,930	3	Array: OGT 180k Cytosure ISCA v2	A-D
16	22q11.21	18,938,160-21,441,944	2.5	Array: OGT 180k Cytosure ISCA v2	A-D
17	22q11.21	21,076,930-21,441,944	0.37	Array: OGT 180k Cytosure ISCA v2	C-D
18	22q11.21	18,876,605-21,499,494	2.6	Array: OGT 180k Cytosure ISCA v2	A-D
19	22q11.21	18,876,605-21,441,944	2.56	Array: OGT 180k Cytosure ISCA v2	A-D
20	22q11.21	18,875,830-21,441,944	2.56	Array: OGT 180k Cytosure ISCA v2	A-D
21	22q11.21	18,661,699-21,661,435	2.99	Array: OGT 180k Cytosure ISCA v2	A-D
22	22q11.21	18,876,605-21,441,944	2.56	Array: OGT 180k Cytosure ISCA v2	A-D
23	22q11.21	18,890,162-21,441,944	2.552	Array: 180k CytoSure Constitutional v3'	A-D
24	22q11.21	18,890,162-21,457,610	2.567	Array: 180k CytoSure Constitutional v3'	A-D
25	22q11.21	18,890,162-21,857,001	2.967	Array: 180k CytoSure Constitutional v3'	A-D
26	22q11.21	18,661,699-21,457,610	2.796	Array: 180k CytoSure Constitutional v3'	A-D
27	22q11.21	18,890,162- 21,441,944	2.5518	Array: OGT 180k Cytosure ISCA v2	A-D
28	22q11.21	18,818,376-21,661,435	2.8	Array: OGT 180k Cytosure ISCA v2	A-D

Supplementary Table 2.2 – Overview of used screening and diagnostic tests.

Domain of testing	Test	Authors - Reference
Intelligence	Bayley Scales of Infant Development - Second edition Dutch version (BSID-II-NL)	van der Meulen, B. F., Ruiter, S. A. J., Spelberg, H. C. L., & Smrkovsky, M. (2004). *Bayley Scales of Infant Development-II-Nederlandse Versie. Handleiding.* Amsterdam: Harcourt Test Publishers. Bayley, N. (1993). *Bayley scales of infant development-2nd Ed.* San Antonio, TX: Psychological Corporation.
	Wechsler Preschool and Primary Scale of Intelligence Revised Dutch version (WPPSI-R)	*Vander Steene, G., & Bos, A. (1997). WPPSI-R, Wechsler Preschool and Primary Scale of Intelligence. Vlaams-Nederlandse Aanpassing, Handleiding. Lisse: Swets & Zeitlinger.* Wechsler, D. (1989). *Wechsler Preschool and Primary Scale of Intelligence-Revised.* San Antonio, TX: The Psychological Corporation.
	Snijders-Oomen Nonverbal test Revised Dutch version (SON-R)	Tellegen, P., Winkel, M., Wijnberg-Williams, B. J., & Laros, J. A. (1998). *Snijders-Oomen Niet-verbale Intelligentietest SON-R 2½-7. Handleiding en Verantwoording.* Boom Testuitgevers.
	Wechsler Preschool and Primary Scale of Intelligence - Third Edition Dutch version (WPPSI-III-NL)	Wechsler, D., Hendriksen, J., & Hurks, P. (2009). WPPSI-III-NL. Nederlandstalige bewerking. Afname en Scoringshandleiding. Amsterdam: Pearson Assessment and Information B.V. Wechsler, D. (2002). *Wechsler Preschool and Primary Scale of Intelligence - Third Edition.* San Antonio, TX: Harcourt Assessment.
	Wechsler Intelligence Scale for Children Third Edition Dutch version (WISC-III-NL)	Kort, W., Schittekatte, M., Dekker, P. H., Verhaeghe, P., Compaan, E. L., Bosmans, M., & Vermeir, G. (2005). WISC-III-NL Wechsler Intelligence Scale for Children. David Wechsler. Derde Editie NL. Handleiding en Verantwoording. Amsterdam: Harcourt Test Publishers. Amsterdam: NIP Dienstencentrum. Wechsler, D. (2002). Wechsler Preschool and Primary Scale of Intelligence - Third Edition. San Antonio, TX: Harcourt Assessment.
	Wechsler Intelligence Scale for Children Fifth Edition Dutch version (WISC-V-NL)	Wechsler, D. (2018). WISC-V-NL. Wechsler Intelligence Scale for Children - Fith Edition - Nederlandstalige bewerking. Afname- en scoringshandleiding. Amsterdam: Pearson.

		Wechsler, D. (2014). Wechsler intelligence scale for children (5th ed.): WISC-V. Bloomington, MN: Pearson.
Language	Clinical Evaluation of Language Fundamentals Dutch version (CELF-4-NL)	Kort, W., Compaan, E. L., Schittekatte, M., & Dekker, P. H. (2010). *Clinical evaluation of language fundamentals (CELF–4–NL) Nederlandse versie. Handleiding [CELF–4 Dutch adaptation manual]*. Amsterdam, the Netherlands: Pearson. Paslawski, T. (2005). The Clinical Evaluation of Language Fundamentals, Fourth Edition (CELF-4). *Canadian Journal of School Psychology*, *20*(1–2), 129–134. https://doi.org/10.1177/0829573506295465
	Clinical Evaluation of Language Fundamentals - Preschool – Second Edition. Dutch version (CELF-P2-NL)	de Jong, J. (2012). *Clinical Evaluation of Language Fundamentals - Preschool – Second Edition. Nederlandstalige Versie. Handleiding [CELF-P2-NL: Dutch Adaptation Manual]*. Amsterdam, the Netherlands: Pearson. Semel, E., Wiig, E. M., & Secord, W. A. (2004). *Clinical Evaluation of Language Fundamentals – Preschool – Second Edition*. San Antonio, TX: The Psychological Corporation.
	N-CDI's: lijsten voor Communicatieve Ontwikkeling. (MacArther Communicative Development Inventories) Dutch version	Zink, I., & Lejaegere, M. (2002). *N-CDI's: lijsten voor Communicatieve Ontwikkeling. Aanpassing en hernormering van de MacArthur CDI's van Fenson et al.* Leuven/Leusden: Acco. Fenson, L., Dale, P. S., Reznick, J. S., Thal, D., Bates, E., Hartung, J. P., … Reilly, J. S. (1993). *MacArthur Communicative Development Inventories: User's guide and technical manual*. San Diego: Singular Publishing Group, Inc.
	Reynell Developmental Language Scales Dutch version (RTOS)	Schaerlaekens, A., Zink, I., & Van Ommeslaeghe, K. (2003). *Reynell Taalontwikkelingsschalen (RTOS). Handleiding: tweede versie*. Lisse: Swets en Zeitlinger. Reynell, J., & Gruber, C. (1990). *Reynell Developmental Language Scales*. Los Angeles: Western Psychological Services.
	Schlichting Test for Language Production Dutch version (STTP)	Schlichting, J. E. P. ., & Lutje Spelberg, H. C. (2010). *Schlichting Test voor Taalproductie-II; Handleiding*. Houten: Bohn Stafleu van Loghum.
	Peabody Picture Vocabulary Test- Third Edition – Dutch version (PPVT-III-NL)	Schlichting, L. (2005). *Peabody Picture Vocabulary Test-III-NL Nederlandse versie Handleiding [PPVT-III-NL Dutch edition Manual)*. Amsterdam: Harcourt Test Publishers. Dunn, L. M., & Dunn, L. (1997). *Manual for the Peabody Picture Vocabulary Test, 3rd ed. (PPVT-III)*. Circle Pines, MN: American Guidance Services.

	Children's Communication Checklist – Second Edition – Dutch version (CCC-2-NL)	Geurts, H. (2007). *CCC-2-NL Children's Communication Checklist – Second Edition – Nederlandse Versie [CCC-2- NL: Dutch Edition. Manual]*. Amsterdam, the Netherlands: Pearson Assessment and Information B.V. Bishop, D. V. M. (2003). *The Children's Communication Checklist-2*. London: Psychological Corporation.
	Renfrew language scales Dutch version (RTNA)	Jansonius, K., Ketelaars, M., Borgers, M., Van Den Heuvel, E., Roeyers, H., Manders, E., & Zink, I. (2014). *Renfrew Taalschalen Nederlandse Aanpassing - Handleiding [Renfrew Language Scales - Dutch Adaptation Manual]*. Antwerpen: Garant. Renfrew, C. (1997). *The Renfrew language scales - manual*. Bicester: Speechmark Publishing Ltd.
	Dutch version of the Nonspeech test (NNST)	Zink, I., & Lembrechts, D. (2000). *NNST: Nederlandstalige Nonspeech Test*. Leuven/Leusden: Acco. Huer, M. B. (1988). *The nonspeech test for receptive/expressive language*. Lake Zurich, III.: Don Johnston Development Equipment.
Visual-Motor skills	Movement Assessment Battery for Children - Second Edition (Movement ABC-2)	Smits-Engelsman, B. (2010). *Nederlandse Bewerking van de Movement ABC-2 NL | Movement Assessment Battery for Children. Handleiding*. Lisse: Swets en Zeitlinger Test Publishers. Henderson, S. E., Sugden, D. A., & Barnett, A. (2007). *Movement Assessment Battery for Children - Second Edition (Movement ABC-2)*. London, UK: The Psychological Corporation.
	Test of Gross Motor Development-Second Edition (TGMD-2)	Ulrich, D. A. (2000). *Test of Gross Motor Development. Second Edition*. Austin Texas: PRO-ED.
	Bruininks-Oseretsky Test of Motor Proficiency - Second Edition (BOT-2)	Bruininks, R. H., & Bruininks, B. D. (2005). *Bruininks-Oseretsky Test of Motor Proficiency (BOT-2) - Second Edition*. London, UK: Pearson.
	The Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI-Beery)	Beery, K. E., Buktenica, N. A., & A., B. N. (2010). *The Beery–Buktenica Developmental Test of Visual–Motor Integration: Administration, scoring, and teaching manual (6th ed.)*. Minneapolis, MN: Pearson.
	Peabody Developmental Motor scales – Second Edition (PDMS-2)	Folio, R. M., & Fewell, R. R. (2000). *Peabody Developmental Motor Scales. Second Edition (PDMS–2) Examiner's Manual*. Austin Texas: PRO-ED.
	L-94 visual perceptual battery	Stiers, P., Van Den Hout, B., Haers, M., Vanderkelen, R., de Vries, L., van Nieuwenhuizen, O., & Vandenbussche, E. (2001). The variety of visual perceptual impairments in pre-school children with

		perinatal brain damage. *Brain Development*, *23*(5), 333– 348. https://doi.org/10.1016/s0387-7604(01)00241-8
	NEPSY-II-NL Dutch version	Zijlstra, H. P., Kingma, A., Swaab, H., & Brouwer, W. H. (2010). *NEPSY-II-NL Nederlandstalige bewerking. Afnamehandleiding.* Amsterdam: Pearson Assessment and Information B.V. Korkman, M., Kirk, U., & Kemp, S. (2007). *NEPSY Second Edition (NEPSY-II).* San Antonio, TX: Harcourt Assessments.
	Children's Visual Impairment Test Dutch version (CVIT)	Vancleef, K., Janssens, E., Petré, Y., Wagemans, J., & Ortibus, E. (2020). An assessment tool for visual perception deficits in Cerebral Visual Impairment: reliability and validity. *Dev Med Child Neurol*, *62*, 119–125. Vancleef, K., Petré, Y., Janssens, E., Bäumer, S., Ortibus, E., & Wagemans, J. (2017). *CVIT 3-6 - Screening test for cerebral impairment in young children.*
	Preschool Judgement of Line Orientation (PJLO)	Benton, A. L., Hamsher, K., Varney, N., & Spreen, O. (n.d.). *Contributions to Neuropsychological Assessment: A Clinical Manual.* New York: Oxford.
	Test of visual-perceptual skills (non-motor) – Revised Dutch version (TVPS-R)	Gardner, M. (1996). *TVPS-R: Test of visual-perceptual skills (non-motor) – Revised.* San Francisco: Psychological and Educational Publication, Inc. Martin, N. A. (2006). *Manual of the Test of Visual Perceptual Skills (TVPS-3). 3rd Edition.* Novato CA: Academic Therapy Publications.
Behaviour	Vragenlijst voor Inventarisatie van Sociaal gedrag voor Kinderen Dutch version (VISK)	Luteijn, E., Minderaa, R., & Jackson, S. (2007). VISK Handleiding. Vragenijst voor Inventarisatie van Sociaal gedrag van Kinderen. Lisse: Swets & Zeitlinger.
	Child Behavior Checklist Dutch version (CBCL)	Verhulst, F., van der Ende, J., & Koolhans, M. (2001). *Child Behavior Checklist voor kinderen van 6 tot 18 jaar (CBCL 6 - 18).* Rotterdam: Erasmus MC - Sophia Kinderziekenhuis. Achenbach, T. M. (2011). Child Behavior Checklist. In *Encyclopedia of Clinical Neuropsychology* (pp. 546–552). New York, NY: Springer New York. https://doi.org/10.1007/978-0-387-79948-3_1529

	Teacher's Report Form Dutch version (TRF)	Verhulst, F. C., & Van der Ende, J. (2013). *Handleiding ASEBA-Vragenlijsten voor leeftijden 6 t/m 18 jaar: CBCL/6-18, YSR en TRF.* Rotterdam: ASEBA Nederland. Achenbach, T. M., & Rescorla, L. A. (2001). *Manual for the ASEBA School-Age Forms & Profiles.* Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.
Autism	Social Responsiveness Scale – Dutch Version (SRS-NL)	Constantino, J. N., & Gruber, C. P. (2005). *Social Responsiveness Scale (SRS).* Los Angeles, CA: Western Psychological Services. Roeyers, H., Thys, M., Druart, C., De Schryver, M., & Schittekatte, M. (2011). *SRS: Screeningslijst voor autismespectrumstoornissen.* Amsterdam, the Netherlands: Hogrefe.
	Autism Diagnostic Observation Schedule Dutch version (ADOS-2)	de Bildt, A., de Jonge, M., & Graeves-Lord, K. (2013). Autism Diagnostic Observation Schedule - Second Edition (ADOS-2). Nederlandse bewerking. Autisme diagnostisch observatieschema. Amsterdam, Nederland: Hogrefe Uitgevers B.V. Lord, C., Rutter, M., DiLavore, P. C., Risi, S., Gotham, K.,& Bishop, S. L. (2012). Autism Diagnostic Observation Schedule, modules 1–4 (2nd ed.). Torrance, CA: Western Psychological Services.
	Autism Diagnostic Interview, Revised (ADI-R)	de Jonge, M., Graeves-Lord, K., & de Bildt, A. (2003). Autism Diagnostic Interview, Revised (ADI-R). Nederlandse bewerking. Autisme diagnostisch interview - revised. Amsterdam, Nederland: Hogrefe Uitgevers B.V. Rutter, M., Le Couteur, A., & Lord, C. (2003). Autism Diagnostic Interview, Revised (ADI-R). Torrance, California: Western Psychological Services.
Attention	ADHD Vragenlijst Dutch version (AVL)	Scholte, & Van der Ploeg. (2005). *Handleiding ADHD-vragenlijst.* Houten: Bohn Stafleu Van Loghum.
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Chapter 3

Chapter 3 - Parent-Reported Social-Communicative Skills of Children with 22q11.2 Copy Number Variants and Siblings

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Abstract

22q11.2 deletion (22q11.2DS) and 22q11.2 duplication (22q11.2Dup) confer risk for neurodevelopmental difficulties, but the characterisation of speech-language and social skills in 22q11.2Dup is still limited. Therefore, this study aims to delineate social-communicative skills in school-aged children with 22q11.2Dup (n = 19) compared to their unaffected siblings (n = 11) and age-matched children with 22q11.2DS (n = 19). Parents completed two standardised questionnaires: the Children's Communication Checklist (CCC-2), screening speech, language, and social skills, and the Social Responsiveness Scales (SRS-2), assessing deficits in social behaviour. Parents report that both children with 22q11.2Dup and 22q11.2DS show more social-communicative deficits than the general population; children with 22q11.2Dup seem to take an intermediate position between their siblings and children with 22q11.2DS. Compared to 22q11.2DS, they demonstrate less frequent and less severe problems, and more heterogeneous social-communicative profiles, with fewer restricted interests and repetitive behaviours. In siblings of 22q11Dup, milder social-communicative difficulties and equally heterogeneous profiles are reported, which might indicate that -in addition to the duplication- other factors such as the broader genetic context play a role in social-communicative outcomes.

3.1 Introduction

Recurrent copy number variants (CNVs) are associated with a significant risk for neurodevelopmental disorders (NDDs), including speech, language, and communication impairments, and social and behavioural difficulties (Chawner et al., 2019; Deshpande & Weiss, 2018; Grayton et al., 2012; Lee & Lupski, 2006; Zarrei et al., 2019). Language and speech are essential for human interaction and communication. Due to their link to and comorbidity with cognition, behaviour, and socio-emotional development, they constitute fundamental research topics. Furthermore, they interact with academic achievement and quality of life measures and may be useful for identifying autistic traits (Carpenter & Drabick, 2011; Nudel et al., 2020; Van Agt et al., 2011; Vyshedskiy et al., 2017). Studies exploring communication skills should address different aspects, such as speech (e.g., articulation of words), structural language (e.g., formulation of sentences), pragmatic language (e.g., use of language in social contexts), and related social components (e.g., social motivation) (Geurts & Embrechts, 2008; Norbury et al., 2004).

Recurrent CNVs at chromosomal locus 22q11.2 are among the most common rare genetic disorders that confer significant risk for NDDs across the lifespan, in particular 22q11.2 deletion syndrome (22q11.2DS) and 22q11.2 duplication (22q11.2Dup). To date, social-communicative skills have been thoroughly studied in 22q11.2DS, confirming that both structural and pragmatic language skills may be profoundly affected in receptive as well as expressive language domains (Solot et al., 2019; Van Den Heuvel, Manders, et al., 2018). Regarding the social and behavioural profile in 22q11.2DS, high rates of autistic features have been described, with an estimated prevalence of autism spectrum disorder (ASD) in 20–42% (Antshel et al., 2007; Fine et al., 2005; Jalal et al., 2021; Van Den Heuvel, Jonkers, et al., 2018).

The question arises whether a duplication in the same chromosomal region will have a similar impact on social-communicative outcomes. Features of 22q11.2Dup are variable, although it is in general associated with a milder phenotype compared to the 22q11.2DS. Physical features include dysmorphism, transient hearing impairment, nutritional problems, cardiovascular defects, growth retardation, and hypotonia, but all at lower rates compared to the 22q11.2DS (Verbesselt et al., 2022). The developmental phenotype is generally characterised by speech-language and motor delays, cognitive impairments, and behavioural problems. Although many case reports describe speech-language

delays, impairments, or behavioural problems in patients with 22q11.2Dup, only a few studies have investigated these problems (Portnoï, 2009; Van Campenhout et al., 2012; Verbesselt et al., 2022; Woodward et al., 2019; Yu et al., 2019).

Regarding the social and behavioural profile, Wenger et al. (2016) used direct instruments and parental questionnaires, such as the Social Responsiveness Scale (SRS) (Constantino & Gruber, 2005), to characterise the neuropsychiatric functioning in children with 22q11.2Dup, compared to children with 22q11.2DS, children with ASD and typically developing children. Consistent with the results of other studies (Clements et al., 2017; Drmic et al., 2022; Lin et al., 2020), mean total SRS scores met clinical cut-offs for mild–moderate social responsiveness deficits in probands with 22q11.2Dup. Based on this study and in agreement with the results from Verbesselt et al. (2022), the estimated prevalence of ASD in 22q11.2Dup is 14–25%, with another third of the sample showing autistic features.

Up to now, no studies have focused on the communication profile in children with 22q11.2Dup, and only some case reports describe the presence of speech-language problems (Portnoï, 2009; Van Campenhout et al., 2012; Verbesselt et al., 2022; Woodward et al., 2019; Yu et al., 2019). Therefore, the purpose of this study is to characterise social-communicative behaviours in school-aged children with 22q11.2Dup. Standardised screening instruments completed by parents are seen as a suitable starting point for collecting data on social-communicative behaviours because parents are reliable informants regarding the abilities of their children (Bennetts et al., 2016; Bishop & McDonald, 2009; Garibaldi et al., 2021; Van Roy et al., 2010). In addition, standardised questionnaires, such as SRS-2 and Children's Communication Checklist (CCC-2), have normed references and therefore enable comparisons with typically developing peers in the general population (Bishop, 2016; Constantino & Gruber, 2012).

To further contribute to the characterisation of syndrome-specific features in children with 22q11.2Dup, two relevant control groups were included. The first control group consists of full-biological unaffected siblings of children with 22q11.2Dup, providing insight into genetic and environmental background factors that may modulate language, cognitive and behavioural outcomes in children with 22q11.2Dup. Including siblings as a control group reduces the impact of contextual factors such as socioeconomic status and educational attainment of the parents. The second control group consists of age-matched

children with 22q11.2DS, enabling pairwise cross-CNV comparisons in a cross-sectional research design. Comparing children with reciprocal CNVs allows us to investigate whether CNVs within the same chromosomal locus have a similar impact on the phenotype or whether changes in gene dosage are associated with mirror phenotypes, as was reported for the 16p11.2 locus (Jacquemont et al., 2011). Additionally, cross-CNV comparisons may contribute to the identification of syndrome-specific social-communicative features (Mervis, 2004).

The current study has a three-fold objective. First, the skills of children with 22q11.2Dup, their siblings, and children with 22q11.2DS will be compared to the norm group scores in the general population. We expect the scores of children with 22q11.2 CNVs to differ from the norm group scores, whereas scores of siblings are expected to be within the same range as the norm group scores. Second, the skills of children with the 22q11.2Dup will be compared to those of their unaffected siblings and of age-matched children with 22q11.2DS. We hypothesise that children with 22q11.2Dup will take an intermediate position between their siblings and children with 22q11.2DS, meaning that they will probably display better social-communicative skills than children with 22q11.2DS and worse than their siblings. Finally, parental reports of *de novo* and familial duplications will be compared to elucidate the influence of the inheritance pattern on social-communicative skills. Likewise, sex differences and differences depending on the country of residence or comorbid ASD diagnosis will be explored.

3.2 Materials and methods

3.2.1 Participants

This prospective study includes 49 participants, consisting of 19 unrelated children with 22q11.2Dup, 19 unrelated children with 22q11.2DS, and 11 unrelated unaffected siblings of the children with 22q11.2Dup. All participants were school-aged children between 6 and 16 years. We only included monolingual Dutch-speaking children or children who had received at least 3 years of full-time Dutch education to mitigate the impact of multilingualism on language development (Cummins, 2000; De Houwer, 2021; Kohnert et al., 2021). Prematurity was an exclusion criterium (i.e., gestational age < 37 weeks) due to the known influence on language development (Barre et al., 2011; Crosbie et al., 2011). Additional exclusion criteria were no language output on the sentence level and severe sensorimotor problems such as severe hearing loss (≥55 dB HL) or severe visual impairments, except for cerebral visual impairment (CVI).

Children with comorbid NDDs such as CVI, ASD, and ADHD were not excluded from the sample because of the high comorbidity, and in the case of ASD, traits may differ between children with ASD with and without underlying genetic defects (Bruining et al., 2010). Finally, participants with more than one (likely) pathogenic CNV were excluded.

Using a genetic-first approach, all children with 22q11.2Dup and 22q11.2DS had a confirmed diagnosis based on the fluorescent in situ hybridisation technique (FISH) or microarray (array CGH). The majority of children with 22q11.2Dup carry the most common 3 Mb microduplication, located at LCR22A-LCR22D (Supplementary table 3.1). One child had breakpoints situated at LCR22A-LCR22B, one at LCR22A-LCR22E, one at LCR22A-LCR22H, one at LCR22B-LCR22C, and one at LCR22C-LCR22D. All children with 22q11.2DS carry the LCR22A-LCR22D microdeletion. All children with 22q11.2 CNVs were index patients diagnosed in a clinical setting because of developmental or medical issues or a combination of both. Due to ethical considerations, siblings did not undergo genetic testing unless there was an indication to do so. However, even in familial cases, there was no indication for referral for genetic testing in siblings.

Table 3.1 shows demographic and clinical data for the three groups of children. Data on developmental milestones and education were obtained from digital medical records or anamnesis provided by parents. Speech-language milestones were delayed in 79% of children with 22q11.2Dup and 95% of children with 22q11.2DS. Speech-language therapy has been received by 84% of children with 22q11.2Dup, all children with 22q11.2DS, and 27% (3/11) of siblings. While all siblings follow regular education, 63% of children with 22q11.2Dup and 74% of those with 22q11.2DS attend special education.

Table 3.1 – Demographic and clinical characteristics across groups.

	22q11.2DS	**22q11.2Dup**	**Siblings of Dup**
Sample Size (*n*)	19	19	11
Sex (*n*, %)			
Male	14 (74%)	10 (53%)	4 (36%)
Female	5 (26%)	9 (47%)	7 (64%)
Chronological age (yrs.mo)			
Average (*SD*)	10.7 (2.5)	10.7 (2.5)	10.10 (2.10)
Median	11.2	11	11
Range	6.7–14.4	6.8–14.9	6.3–16.1

Country of residence (*n*, %)			
Belgium	15 (79%)	10 (53%)	5 (45%)
The Netherlands	4 (21%)	9 (47%)	6 (55%)
Type of education (*n*, %)			
Special education	14 (74%)	12 (63%)	0 (0%)
Regular education	5 (26%)	7 (37%)	11 (100%)
Speech-language delays (*n*, %)	18 (95%)	15 (79%)	0 (0%)
Speech-language therapy (*n*, %)	19 (100%)	16 (84%)	3 (27%)
Formal NDD diagnoses (*n*, %)			
ASD	8 (42%)	2 (11%)	0 (0%)
ADHD	4 (21%)	4 (21%)	0 (0%)
SLD	1 (5%)	4 (21%)	0 (0%)
DCD	0 (0%)	4 (21%)	0 (0%)
DLD	0 (0%)	3 (16%)	0 (0%)
CVI	0 (0%)	3 (16%)	0 (0%)
Inheritance pattern (*n*, %)			
De novo	18 (95%)	8 (42%)	
Inherited	1 (5%)	8 (42%)	/
Unknown	0 (0%)	3 (16%)	

Note. Abbreviations: NDD, neurodevelopmental disorders; ASD, autism spectrum disorder; ADHD, attention deficit/hyperactivity disorder; SLD, specific learning disorder; DLD, developmental language disorder; CVI, cerebral visual impairment; DCD, developmental coordination disorder.

3.2.2 Research design

All participants were recruited through the Centre for Human Genetics of UZ Leuven or Maastricht University Medical Centre. Questionnaires were provided and completed through the online platform Qualtrics. Data were prospectively collected during home visits or consultations at the hospital from 2012 to 2022. A subgroup of children with 22q11.2DS and 8 children with 22q11.2Dup has previously been published (Van Den Heuvel et al., 2017; Van Den Heuvel, Jonkers, et al., 2018; Verbesselt et al., 2022).

A cross-sectional study design with pairwise comparisons was applied. The first pairwise comparison consisted of CNV pairs, for which 19 children with 22q11.2DS were matched to children with 22q11.2Dup on chronological age (CA), reducing the impact of age-related advantages such as having more experience in social interactions. Age matching was within 0.5 years of age, with an average deviation of 3 months. No significant differences between groups were found for the matching parameter using paired samples Student's *t*-test (t =

0.129, $p = 0.899$) (Mervis, 2004). The second pairwise comparison was aimed at intrafamilial pairs, consisting of children with 22q11.2Duplication and their unaffected siblings. Only 11 children with 22q11.2Dup had a sibling willing to participate who met the criteria of age, at term birth, and no neurological defects. In families with more than one sibling, the sibling closest in age to the child with the 22q11.2Dup was selected.

3.2.3 Measurements

Children's social-communicative skills were investigated by means of two standardised parental questionnaires. The first one is the Dutch edition of the Children's Communication Checklist-Second version (CCC-2) (Bishop, 2003; Geurts, 2007), a 70-item screening instrument, used to assess a wide scope of everyday communicative skills, including speech, structural and pragmatic language skills, and social abilities. Parents need to indicate how often their child shows certain communicative behaviour on a frequency scale of 0–3 (0 = less than once a week or never, 3 = several times a day or always). Raw scores can be converted into scaled scores (SS) based on the chronological age (CA) of the participant. The questionnaire is normed for children between 4 and 15.6 years of age. Since one sibling was already 16 years of age, the scores of the oldest norm group were used to convert raw scores to scaled scores.

In total, there are 10 different norm-referenced subscales, each with an average SS of 10 and a standard deviation (*SD*) of 3. The higher the score, the weaker the social-communicative skills; e.g., an SS of 17 on a given subscale is more than two *SD*s above average, implying considerable difficulties within this domain. The first four subscales measure speech and structural language components (A. Speech, B. Syntax, C. Semantics, and D. Coherence), while the next four assess pragmatic language (E. Inappropriate initiation, F. Stereotyped language, G. Use of context, H. Non-verbal communication) and the final two focus on autistic features (I. Social relations and J. Interests). In addition, we will focus on two main composite scores: the General Communication Composite (GCC) and the Pragmatic Composite (PC). The GCC is based on all communication scales (A-H) with a clinical cut-off of 104 points (pc 10) for moderate communication problems and 117 (pc 2) for severe communication deficits, while the PC is the combined score of the four pragmatic subscales, giving an indication of pragmatic language difficulties. The cut-offs for moderate and severe pragmatic problems are scores of 53 (pc 10) and 60 (pc 2) respectively (Bishop, 1998, 2003; Norbury et al., 2004).

The second questionnaire is the Dutch edition of the Social Responsiveness Scales or Social Responsiveness Scales—Second edition (SRS or SRS-2) (Constantino & Gruber, 2012; Roeyers et al., 2015), a 65-item valid screening questionnaire that uses a Likert-scale of 1–4 (1 = not true, 4 = almost always true) to quantify deficits in social behaviour associated with ASD. It contains 5 different treatment subscales: Social Awareness, Social Cognition, Social Communication, Social Motivation, and Restricted Interests and Repetitive Behaviour (Bruni, 2014; Constantino & Gruber, 2005, 2012; Roeyers et al., 2011, 2015). For participants between 4 and 18 years of age, raw scores can be converted to country- and sex-normed *T*-scores, each with an average of 50 and *SD* of 10. The higher the *T*-score, the more social responsiveness problems someone experiences with *T*-scores between 61 and 75 (pc < 16) are interpreted as mild–moderate and above 75 (pc < 1.2) as severe social responsiveness impairments, according to the test manual (Constantino & Gruber, 2012).

Due to the earlier data collection, parents of children with 22q11.2DS have completed the SRS, whereas parents of children with 22q11.2Dup and their unaffected children have filled out the SRS-2. In the Dutch version, there are no differences between SRS and SRS-2 regarding the questions and norms, apart from the addition of two composite scores, Restricted Interests and Behaviour (RIB) and Social Communication and Interaction (SCI), to better correspond with the DSM-5 criteria (American Psychiatric Association, 2013). The RIB is the same as the restricted interests and repetitive behaviour subscale, while the SCI consists of the four other treatment subscales (Bruni, 2014; Constantino & Gruber, 2012). Both composite scores were also derived for children with 22q11.2DS. Henceforth, we will refer to SRS-2 for all groups of children.

3.2.4 Data analysis

Depending on the violation of assumptions, parametric (Student's) or non-parametric (Wilcoxon signed rank) one-sample *t*-tests are applied to investigate whether the skills of children with 22q11.2 CNVs and siblings significantly differ from the norm group scores. Given the expected large intra-group variability in children with 22q11.2 CNVs, traditional statistical testing is combined with descriptive and qualitative analyses using a three-tiered method. Hence, the scores are analysed at three different levels with statistical analyses of group differences, proportion differences across groups, and detailed characterisation of typical or atypical individual patterns (Olsson, 2005). At the group level, CNV pairs (19 age-matched children with 22q11.2Dup and 22q11.2DS) and intrafamilial pairs (11 children with 22q11.2Dup and their

siblings) are statistically compared on the main composite scores of SRS-2 and CCC-2, using pairwise Student's *t*-tests. At the intermediate or subgroup level, the proportions of participants with clinical scores on SRS-2 and CCC-2 composite scores are compared across CNV and intrafamilial pairs using McNemar's test. Clinical cut-off scores were 104 for GCC, 53 for PC, and 60 for SRS scores.

On the subtest level, children were considered to have social-communicative difficulties when their scores deviated more than one *SD* from the norm group average. Consequently, subgroup analyses allow investigating whether individual variations affect the mean value of the group, which is useful in small sample studies with high risks for skewed group results by large intra-group variability. At the individual level, we look at interesting profiles within the group of children with 22q11.2Dup, such as the impact of the inheritance pattern, sex, or country of residence on the social-communicative results, using independent-sample *t*-tests. Finally, we qualitatively investigate the influence of an ASD diagnosis on outcomes. Due to multiple testing, Bonferroni corrections were applied to reduce type I errors. All statistical analyses were performed using JASP version 0.16.3 (JASP Team, 2022) and R 4.2.1 (R core team, 2017; Wickham, 2016).

3.3 Results

3.3.1 CNVs and siblings compared to norm group scores

Figure 3.1 depicts boxplots of the composite scores on CCC-2 and SRS-2 for the three groups of children, with the grey zones indicating how many children have mild–moderate to severe social-communicative problems and the dashed line showing norm group averages. The box plots show a wide range of scores for children with CNVs, especially in the 22q11.2Dup group. One-sample *t*-tests were used to compare the three groups of children to the norm group on all reported composite scores. Results in Supplementary Table 3.2 shows that parental reports of children with 22q11.2Dup significantly differed from the norm group on all CCC-2 and SRS-2 composite scores ($0.001 < p < 0.004$) with moderate to large effect sizes ($d > 1.032, r > 753$). The same results were found in children with 22q11.2DS ($p < 0.001$) with large effect sizes ($d > 1.555$). In both groups, the results remained significant after the Bonferroni correction. In contrast to both CNV groups, no significant differences were found between parental reports of siblings and the norm group on the CCC-2 and SRS-2 composite scores.

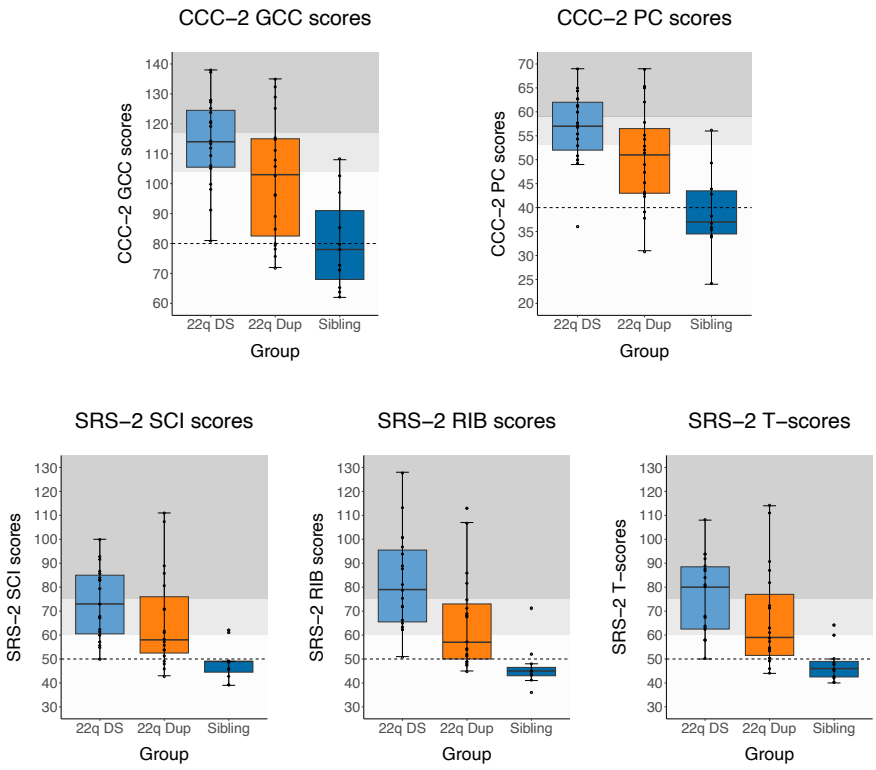

Figure 3.1 – Boxplots for CCC-2 and SRS-2 composite scores across groups. The dashed lines show norm group averages. The grey zones indicate the severity of the problems; the darker the grey, the more severe the difficulties: mild–moderate = light grey zone and severe = darker grey zone, based on clinical cut-off scores for CCC-2 and SRS-2. Abbreviations. GCC, General Communication Composite (norm group average = 80, cut-off: >104 = mild–moderate (pc 10), ≥117 = severe (pc 2)); PC, Pragmatic Composite (norm group average = 50, cut-off: >53 = mild–moderate (pc 10), ≥60 = severe (pc 2)); SCI, Social Communication and Interaction, RIB, Repetitive interests and behaviour, Total (norm group average = 50, cut-off: >60 = mild–moderate (pc 16), ≥76 severe (pc 1.8)).

3.3.2 Cross-CNV and intrafamilial comparisons at group level: Mean differences

Mean composite scores in Table 3.2 illustrate mild–moderate to severe reported social-communicative difficulties across all composite scores in children with 22q11.2DS, without reported social-communicative difficulties in children with 22q11.2Dup and their siblings, except for mean SRS-2 scores in the 22q11.2Dup group. Parametric paired *t*-tests were used to perform cross-CNV and intrafamilial comparisons. Pairwise cross-CNV comparisons revealed

significantly weaker GCC, PC, and RIB scores in children with 22q11.2DS compared to children with 22q11.2Dup with moderate effect sizes. Additionally, pairwise intrafamilial comparisons showed significantly weaker scores across all CCC-2 and SRS-2 composite scores in children with 22q11.2Dup compared to their siblings with moderate to large effect sizes. However, all former significant results did not survive Bonferroni correction.

At the subtest level, box plots in Figure 3.2 show similar distributions across all subtests, indicating that children with 22q11.2Dup mostly show weaker scores than their siblings and better scores than children with 22q11.2DS. For children with 22q11.2DS, mean subtest scores are within the clinical range across all subtests, while children with 22q11.2Dup only show clinical scores for SRS-2 subtests Cognition, Communication, Motivation, and Restricted Interests/Repetitive Behaviours (Supplementary Table 3.3). Mean subtest scores of siblings are within the normal range across all subtests.

Table 3.2 – Mean composite results on CCC-2 and SRS-2 for cross-CNV and intrafamilial comparisons.

	22q11.2Dup (*n* = 19)	**22q11.2DS (*n* = 19)**	***t*-test *t* =, *p* =, *d* =**	**22q11.2Dup (*n* = 11)**	**Siblings Dup (*n* = 11)**	***t*-test *t* =, *p* =, *d*=**
CCC-2 GCC Mean (*SD*) Range	101.58 (20.29) 72.00–135.00	114.12 (14.99) 81.00–138.00	*t* = -2.281 *p* = 0.035 * *d* = -0.523	98.36 (19.67) 72.00–132.00	80.55 (16.01) 62.00–108.00	*t* = 2.647 *p* = 0.024 * *d* = 0.798
CCC-2 PC Mean (*SD*) Range	50.58 (10.25) 31.00–69.00	56.63 (7.59) 36.00–69.00	*t* = -2.190 *p* = 0.042 * *d* = -0.502	49.27 (8.79) 38.00–65.00	39.09 (8.57) 24.00–56.00	*t* = 3.136 *p* = 0.011 * *d* = 0.946
SRS-2 SCI Mean (*SD*) Range	66.05 (20.07) 43.00–111.00	73.47 (15.09) 50.00–100.00	*t* = -1.313 *p* = 0.206 *d* = -0.301	58.18 (13.00) 43.00–89.00	48.36 (7.50) 39.00–62.00	*t* = 2.276 *p* = 0.046 * *d* = 0.686
SRS-2 RIB Mean (*SD*) Range	65.53 (19.94) 45.00–113.00	81.53 (19.69) 51.00–128.00	*t* = -2.282 *p* = 0.035 * *d* = -0.523	57.27 (12.33) 45.00–86.00	46.64 (9.00) 36.00–71.00	*t* = 2.241 *p* = 0.049 * *d* = 0.676
SRS-2 Total Mean (*SD*) Range	66.79 (21.06) 44.00–114.00	76.05 (16.28) 50.00–108.00	*t* = -1.508 *p* = 0.149 *d* = -0.346	58.46 (13.46) 44.00–91.00	47.82 (7.79) 40.00–64.00	*t* = 2.292 *p* = 0.045 * *d* = 0.691

Note. Alpha = 0.05 *; alpha after Bonferroni correction = 0.01. **. GCC, General Communication Composite (norm group average = 80, cut-off: >104 = mild–moderate, ≥117 = severe); PC, Pragmatic Composite (norm group average = 40, cut-off: >53 = mild–moderate, ≥60 = severe); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour, Total (norm group average = 50, cut-off: >60 = mild–moderate, ≥76 severe).

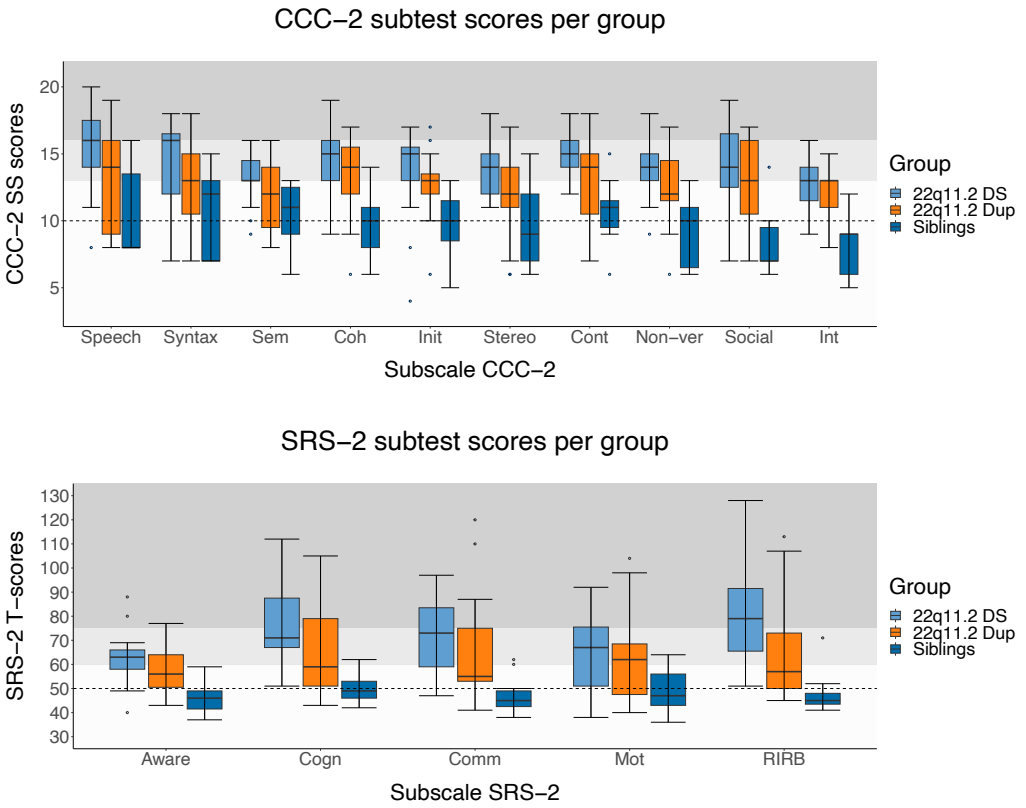

Figure 3.2 – Boxplots for CCC-2 and SRS-2 group scores across subtests. The dashed lines show norm group averages. The grey zones indicate the severity of the problems; the darker the grey, the more severe the difficulties: mild–moderate = light grey zone and severe = darker grey zone. Abbreviations: CCC-2 subtests ($M = 10$, $SD = 3$): Speech; Syntax; Sem, Semantics; Coh, Coherence; Init, Inappropriate Initiation; Stereo, Stereotyped Language; Cont, Use of Context; Non-ver, Non-verbal Communication; Social, Social relations; Int, Interests. SRS-2 subtests ($M = 50$, $SD = 10$): Aware, Social Awareness; Cogn, Social Cognition; Comm, Social Communication; Mot, Social Motivation; RIRB, Restricted Interests and Repetitive Behaviours.

3.3.3 Cross-CNV and intrafamilial comparisons at subgroup level: proportion differences

Based on the CCC-2, general communication (GCC) difficulties have been reported in 47% (9/19) of children with 22q11.2Dup, 9% (1/11) of their siblings, and 79% (15/19) of children with 22q11.2DS (see Table 3.3). Although the reported proportions of GCC difficulties differ across groups, both intrafamilial and cross-CNV pairwise comparisons were not found to be significant according to McNemar's test. Similarly, parents reported pragmatic problems (PC) in 37% (7/19) of children with 22q11.2Dup, 9% (1/11) of their siblings, and 68% (13/19) of children with 22q11.2DS without any significant differences for intrafamilial and cross-CNV comparisons. At the subtest level, the most commonly reported problems in children with 22q11.2Dup were problems with Speech in 58% (11/19) and Use of Context and Coherence in 53% (10/19), while in their siblings, the most commonly reported difficulties were difficulties with Speech in 27% (2/11), and Syntax and Coherence in 18% (2/11%) (Supplementary Table 3.3). As in children with 22q11.2Dup, the most common concerns in children with 22q11.2DS were problems with Speech and Use of Context in 79% (15/19) and Coherence in 68% (13/19).

Based on the SRS-2, total social responsiveness problems have been reported in 47% (9/19) of children with 22q11.2Dup, 9% (1/11) of their siblings, and 79% (15/19) of children with 22q11.2DS. Proportions of difficulties on the composite scores are displayed in Table 3.3. Intrafamilial pairwise comparisons did not reveal any statistical differences between children with 22q11.2Dup and their siblings for SRS-2 composite scores. Neither did cross-CNV pairwise comparisons for SRS-2 SCI ($p = 0.131$) or total composite score ($p = 0.077$). However, significantly more children with 22q11.2DS (95%) were reported to have RIB difficulties compared to children with 22q11.2Dup (47%, $p = 0.016$), but the results did not remain significant after Bonferroni correction. At the subtest level, the most commonly reported difficulties in children with 22q11.2Dup were problems with Social Motivation in 53% (10/19) and Social Communication and Restricted Interests/Repetitive Behaviours in 47% (9/19), while Social Motivation is the most commonly reported problem in their siblings in 18% (2/11). In children with 22q11.2DS, the most commonly reported difficulties were Restricted Interests/Repetitive Behaviours in 95% (18/19), problems with Social Cognition in 89% (17/19), and Social Communication in 68% (13/19).

Table 3.3 – Proportions of children with reported difficulties across composite scores on CCC-2 and SRS-2.

	22q11.2DS (*n* = 19)	**22q11.2Dup (*n* = 19)**	**Siblings of Dup (*n* = 11)**
CCC-2 GCC	15/19 (79%)	9/19 (47%)	1/11 (9%)
CCC-2 PC	13/19 (68%)	7/19 (37%)	1/11 (9%)
SRS-2 SCI	14/19 (74%)	9/19 (47%)	2/11 (18%)
SRS-2 RIB	18/19 (95%)	9/19 (47%)	1/11 (9%)
SRS-2 Total	15/19 (79%)	9/19 (47%)	1/11 (9%)

Note. GCC, General Communication Composite (cut-off: >104 = mild–moderate problems); PC, Pragmatic Composite (cut-off: >53 = mild–moderate problems); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour, Total (cut-off: >60 = mild–moderate problems).

3.3.4 Within-group comparisons at individual level

Because of the specific interest in social-communication profiles of children with 22q11.2Dup, we analysed certain subgroups in more detail. First, parental reports of children with *de novo* and familial duplications were compared to investigate the influence of the inheritance pattern on the reported social-communicative profile. Qualitatively, parents reported more heterogeneous profiles in children with inherited duplications compared to children with *de novo* duplications on SRS composite scores, but statistical tests failed to find any significant differences (Supplementary Table 3.4). Accordingly, no sex or country differences were found on the composite scores (Supplementary Table 3.5 and 3.6). Qualitatively, girls showed weaker SRS composite scores with higher variability compared to boys, whereas Dutch children from the Netherlands had more heterogeneous profiles with better mean GCC scores compared to Belgian Dutch-speaking children. The two children with 22q11.2Dup and comorbid ASD showed qualitatively weaker SRS composite scores, but CCC composite scores were in line with the scores in the overall 22q11.2Dup group.

3.4 Discussion

The purpose of the current study was to characterise social-communicative skills in children with 22q11.2Dup, compared to the profiles of their unaffected siblings and age-matched children with 22q11.2DS. Moreover, we aimed to investigate whether the profiles of these groups differed from norm group profiles. Therefore, two standardised screening instruments, the CCC-2 and SRS-2, were completed by parents. Additionally, the three-tiered method was used to analyse between- and within-group differences in the composite scores

of both questionnaires. Based on parental reports, children with 22q11.2 CNVs experience more social-communicative difficulties compared to their typically developing peers in the general population, which is in agreement with the literature (Lin et al., 2020; Wenger et al., 2016). In contrast, siblings of children with 22q11.2Dup in the current study did not differ from the norm group and may therefore be compared to peers in the general population.

In children with 22q11.2 CNVs, all mean SRS-2 composite scores met clinical cut-offs for mild–moderate to severe social responsiveness concerns, confirming previous research (Clements et al., 2017; Wenger et al., 2016). Comparisons at the group level demonstrated that children with 22q11.2Dup performed weaker on all aspects of social responsiveness compared to their siblings but only better on Restricted Interests/Repetitive Behaviours compared to children with 22q11.2DS. Consequently, the absence of significant differences between 22q11.2 CNVs for SCI and total SRS-2 scores may suggest that, on average, children with 22q11.2Dup have similar levels of social communication and interaction (SCI) problems as children with 22q11.2DS. Mean CCC-2 composite scores, measuring communication challenges, were just below clinical cut-offs in children with 22q11.2Dup, whereas they met clinical cut-offs for mild–moderate communication concerns in children with 22q11.2DS. Pairwise comparisons confirmed that children with 22q11.2Dup show better general communication (GCC) and pragmatic skills (PC) compared to children with 22q11.2DS but weaker overall social-communication skills compared to their siblings. These results might indicate that children with 22q11.2Dup take an intermediate position between their siblings and children with 22q11.2DS regarding communication skills. Moreover, the heterogeneous communication profiles in siblings might suggest that—in addition to the duplication—other factors, such as the broader genetic background and socioeconomic status, play a role in the social-communicative outcomes (De Smedt et al., 2007; Pizzo et al., 2019). Specifically, not all observed features are definitively linked to the CNV alone; they may also result from interactions with environmental influences and broader genetic factors. For example, in patients with familial 22q11.2DS, one of these social factors could be the lower educational attainment level of both parents. This is caused by the CNV in the affected parent and influenced by assortative mating, which often results in a lower educational level in the unaffected parent and could, therefore, also influence the outcomes in the unaffected siblings (De Smedt et al., 2007). Consequently, it is important to consider both the broader genetic and environmental contexts.

Proportion differences at the subgroup level showed that approximately half (47%) of the children with 22q11.2Dup, 9% of their siblings, and most (79%) of the children with 22q11.2DS have general communication (GCC) and social responsiveness difficulties, with slightly lower rates for pragmatic difficulties (PC). Based on the SRS-2, no significant differences were found regarding social communication and interaction (SCI) between both CNV groups, which is in line with the results of Lin et al. (2020). Remarkably, almost all children with 22q11.2DS (95%) show Restricted Interests/Repetitive Behaviours (RIB), which is significantly higher in comparison to children with 22q11.2Dup (47%). However, results must be interpreted with caution because none of these differences remained statistically significant after the Bonferroni correction. Additionally, some of these results contrast with earlier findings (Wenger et al., 2016), stating that children with 22q11.2Dup who demonstrated ASD traits without meeting all criteria for the diagnosis of ASD (n = 15, 4–18 years) exhibit more restricted and repetitive behaviours. Conversely, a study of 100 patients with 22q11.2DS (1–35 years) indicated that children with 22q11.2DS rather show social communication deficits than restricted and repetitive behaviours (Niklasson et al., 2009). However, Lin et al. (2020) found no differences in restricted and repetitive behaviours between 38 patients with 22q11.2Dup (6–61 years) and 106 with 22q11.2DS (5–49 years). These contrasting findings might be partially explained by high rates of ASD in our 22q11.2DS cohort (n = 8) compared to lower rates in the 22q11.2Dup cohort (n = 2); however, only one patient out of 19 with 22q11.2DS did not show restricted interests and repetitive behaviours. Other potential causes are the use of different measurements, different age ranges, and limited sample sizes across these studies.

Another interesting finding in the current study is that parents of children with 22q11.2 CNVs are most concerned about the same communication domains, in particular speech, use of context, and coherence, but consistently to a lesser extent in children with 22q11.2Dup. These similar concerns might suggest overlapping communicative phenotypes in 22q11.2 CNVs. However, it should be mentioned that the nature of these indicated speech problems might be different, with patients with 22q11.2DS showing more structural defects, such as cleft palate, which potentially affects speech outcomes. In contrast, speech problems in 22q11.2Dup might be characterised by more disorder-specific features and influenced by the broader familial context since this was the most reported problem among siblings.

Surprisingly, 84% of children with 22q11.2Dup received speech-language therapy, although parents reported significant communication problems in only 47% of them. Potential explanations for these seemingly contradictory findings are different indications for speech-language therapy, such as SLD in certain children or overestimation of their communication skills by parents. Therefore, direct speech and language assessments in this population may clarify whether rates of reported communication problems are underestimated in children with 22q11.2Dup. Finally, detailed analyses of subgroups in children with 22q11.2Dup showed no fundamental sex differences or differences dependent on the inheritance pattern, country of residence, or comorbid ASD diagnosis.

3.4.1 Strengths, limitations and future

The inclusion of two relevant control groups is a key strength of the current study, suggesting an impact of the duplication in addition to the familial context on the social-communicative phenotype. Moreover, using a standardised instrument guaranteed reliable and valid norm- referenced results. Although parents are seen as reliable informants regarding everyday social- communicative skills, using an indirect approach might introduce bias, such as social desirability, misjudgements, misinterpretation, or even insufficient understanding of the questions. Therefore, questionnaires should be complemented by direct measurements in future studies to confirm the current findings in children with 22q11.2Dup and to further delineate the speech, language, and social communication profiles in this population (Bishop & McDonald, 2009; Garibaldi et al., 2021). Since the results did not control for cognitive abilities in cross-CNV comparisons or for age in intrafamilial comparisons, differences detected in the social-communication profile might be partly attributed to cognitive differences or more experience in everyday social-communicative interactions. Consequently, in addition to direct assessment of language, assessments of cognitive functioning are needed to determine the exact role of this potentially confounding factor.

The use of a genetic-first approach in a clinically ascertained cohort might introduce bias in the observed social-communication profiles and, therefore, not cover the whole spectrum of profiles. More likely, rates of reported problems will be lower in the population of children with 22q11.2Dup, as children without an indication for diagnosis are often not referred for genetic testing. Therefore, future studies should include a third comparison group consisting of carrier siblings diagnosed through segregation analysis. Since

children with 22q11.2 CNVs did not undergo whole genome sequencing, the potential presence of additional pathogenic CNVs or single nucleotide variants might explain differences in phenotypes as well, especially in children with a rather severe phenotype. In addition, not all siblings were genetically tested; therefore, we could not exclude the presence of a CNV with certainty. However, in the remaining families, there was no indication for genetic testing of siblings; they all attended regular education, and there were no concerns regarding their development.

Two final limitations are the small sample in the current study and the heterogeneity of the population, leading to lower statistical power and restricting our ability to draw general conclusions about the whole population of children with 22q11.2Dup. Interestingly, the relatively small sample did not prevent us from finding significant results. Nevertheless, large-scale multicentre studies are needed to further delineate the social-communicative profile in this heterogeneous population. Despite its limitations, this study certainly adds to the characterisation of the social-communicative skills in children with 22q11.2Dup.

3.5 Conclusions

The current study contributes to the understanding of the social-communicative phenotype in children with 22q11.2Dup, in comparison to the profiles of their siblings and age-matched children with 22q11.2DS. These results are important for healthcare professionals across different clinical settings and indicate the need for social-communicative follow-up in children with 22q11.2Dup. Since parents report high rates of social-communicative challenges in children with 22q11.2Dup, healthcare professionals should be aware of the high risk of social-communicative problems and refer to a speech-language pathologist for screening or diagnostic testing. Finally, future research should focus on deep phenotyping of the communication profile of children with 22q11.2Dup using standardised direct language assessments.

3.6 Supplementary material

Supplementary Table 3.1 – Additional genetic information in 22q11.2Dup (*n = 19)*. Chromosomal position according to Hg19, region, size and break points of the duplications.

Patient number	Region	Chromosomal positions - Hg19	Size (Mb)	Break Points
1	22q11.21q11.23	18,844,632-24,977,286	6.1	A-F
2	22q11.21	18,890,162- 21,441,944	2.5518	A-D
3	22q11.21	18,861,748-21,462,353	2.6	A-D
4	22q11.21	18,628,147-22,123,338	3.49	A-D
5	22q11.21	18,875,830-21,441,944	2.6	A-D
6	22q11.21	21,076,930-21,441,944	0.37	C-D
7	22q11.21	NA	NA	NA
8	22q11.21	18,818,376-21,661,435	2.8	A-D
9	22q11.21	NA	NA	NA
10	22q11.21	NA	NA	A-B
11	22q11.21	NA	NA	A-D
12	22q11.21	NA	NA	B-C
13	22q11.21	18,890,162-21,857,001	2.967	A-D
14	22q11.21	NA	NA	A-D
15	22q11.21	NA	2.6	A-D
16	22q11.21q11.22	17,041,724 – 21,289,605	NA	A-D
17	22q11.21	NA	NA	NA
18	22q11.21	NA	NA	A-E
19	22q11.21	NA	NA	A-D

Supplementary Table 3.2 – CNVs and siblings compared to the normative sample

	22q11.2DS (*n* = 19)	**22q11.2Dup (*n* = 19)**	**Siblings of Dup (*n* = 11)**
CCC-2 GCC (*M* = 80)			
Mean (*SD*)	114.12 (14.99)	101.58 (20.29)	80.55 (16.01)
Median	114.00	103.00	78.00
p =	< 0.001**	< 0.001**	0.912
r = / *d* =	2.275	1.064	0.034
CCC-2 PC (*M* = 40)			
Mean (*SD*)	56.63 (7.59)	50.58 (10.25)	39.09 (8.57)
Median	57.00	51.00	37.00
p =	< 0.001**	< 0.001**	0.732
r = / *d* =	2.192	1.032	-0.106
SRS-2 SCI (*M* = 50)			
Mean (*SD*)	73.47 (15.09)	66.05 (20.07)	48.36 (7.50)
Median	73.00	58.00	49.00
p =	< 0.001**	0.002**	0.486
r = / *d* =	1.555	0.811	-0.218
SRS-2 RIB (*M* = 50)			
Mean (*SD*)	81.53 (19.69)	65.53 (19.94)	46.64 (9.00)
Median	79.00	57.00	45.00
p =	< 0.001**	0.004**	0.075
r = / *d* =	1.602	0.753	-0.621
SRS-2 Total (*M* = 50)			
Mean (*SD*)	76.05 (16.28)	66.79 (21.06)	47.82 (7.79)
Median	80.00	59.00	46.00
p =	< 0.001**	0.002**	0.374
r = / *d* =	1.601	0.863	-0.280

Note. Alpha = 0.05 *; alpha after Bonferroni correction = 0.01. **. GCC, General Communication Composite (norm group average = 80, cut-off: >104 = mild–moderate, ≥117 = severe); PC, Pragmatic Composite (norm group average = 40, cut-off: >53 = mild–moderate, ≥60 = severe); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour, Total (norm group average = 50, cut-off: >60 = mild–moderate, ≥76 severe).

Supplementary Table 3.3 – Descriptive statistics CCC-2 and SRS-2 across groups

			22q11.2DS (*n* = 19)	**22q11.2Dup (*n* = 19)**	**Siblings of Dup (*n* = 11)**
CCC-2	Speech				
		Mean (*SD*)	15.37 (3.13)	13.16 (3.86)	10.36 (3.36)
		Median	16.00	14.00	8.00
		Range	8.00 – 20.00	8.00 – 19.00	8.00 – 16.00
		% with problems	78.95%	57.89%	27.27%
	Syntax				
		Mean (*SD*)	14.32 (3.15)	12.37 (3.47)	10.73 (3.20)
		Median	16.00	13.00	12.00
		Range	7.00 – 18.00	7.00 – 18.00	7.00 – 15.00
		% with problems	63.16%	42.11%	18.18%
	Semantics				
		Mean (*SD*)	13.26 (1.91)	12.05 (2.57)	10.46 (2.46)
		Median	13.00	12.00	11.00
		Range	9.00 – 16.00	8.00 – 16.00	6.00 – 14.00
		% with problems	36.84%	42.11%	0.00%
	Coherence				
		Mean (*SD*)	14.53 (2.48)	13.42 (2.87)	9.91 (2.66)
		Median	15.00	14.00	10.00

Range	9.00 – 19.00	6.00 – 17.00	6.00 – 14.00
% with problems	68.42%	52.63%	18.18%
Inappropriate Initiation			
Mean (*SD*)	13.58 (3.15)	12.68 (2.38)	9.82 (2.52)
Median	15.00	13.00	10.00
Range	4.00 – 17.00	6.00 – 17.00	5.00 – 13.00
% with problems	63.16%	26.32%	0.00%
Stereotyped Language			
Mean (*SD*)	14.11 (2.00)	12.16 (3.45)	9.55 (3.08)
Median	14.00	12.00	9.00
Range	11.00 – 18.00	6.00 – 17.00	6.00 – 15.00
% with problems	63.16%	36.84%	9.09%
Use of Context			
Mean (*SD*)	15.11 (1.79)	13.11 (3.31)	10.64 (2.34)
Median	15.00	14.00	11.00
Range	12.00 – 18.00	7.00 – 18.00	6.00 – 15.00
% with problems	78.95%	52.63%	9.09%
Non-verbal Communication			
Mean (*SD*)	13.84 (2.06)	12.63 (2.69)	9.09 (2.59)
Median	14.00	12.00	10.00
Range	9.00 – 18.00	6.00 – 17.00	6.00 – 13.00
% with problems	57.89%	36.84%	0.00%
Social Relations			

	Mean (*SD*)	13.90 (3.14)	12.79 (3.19)	8.36 (2.38)
	Median	14.00	13.00	7.00
	Range	7.00 – 19.00	7.00 – 17.00	6.00 – 14.00
	% with problems	57.89%	47.37%	9.09%
	Interests			
	Mean (*SD*)	12.63 (2.31)	12.05 (2.15)	8.18 (2.23)
	Median	13.00	13.00	9.00
	Range	9.00 – 16.00	8.00 – 15.00	5.00 – 12.00
	% with problems	36.84%	15.79%	0.00%
SRS-2	Social Awareness			
	Mean (*SD*)	62.16 (10.36)	58.05 (10.45)	46.46 (7.22)
	Median	63.00	56.00	46.00
	Range	40.00 – 88.00	43.00 – 77.00	37.00 – 59.00
	% with problems	52.63%	36.84%	0.00%
	Social Cognition			
	Mean (*SD*)	77.21 (15.04)	66.00 (19.50)	50.00 (6.03)
	Median	71.00	59.00	49.00
	Range	51.00 – 112.00	43.00 – 105.00	42.00 – 62.00
	% with problems	89.47%	42.11%	9.09%
	Social Communication			
	Mean (*SD*)	72.21 (15.25)	65.68 (21.90)	47.27 (7.76)
	Median	73.00	55.00	45.00
	Range	47.00 – 97.00	41.00 – 120.00	38.00 – 62.00

% with problems	68.42%	47.37%	9.09%
Social Motivation			
Mean (*SD*)	64.37 (16.14)	62.42 (18.27)	49.00 (9.87)
Median	67.00	62.00	47.00
Range	38.00 – 92.00	40.00 – 104.00	36.00 – 64.00
% with problems	57.89%	52.63%	18.18%
Restricted Interests and Repetitive Behaviours			
Mean (*SD*)	81.53 (19.69)	65.53 (19.94)	47.73 (8.28)
Median	79.00	57.00	45.00
Range	51.00 – 128.00	45.00 – 113.00	41.00 – 71.00
% with problems	94.74%	47.37%	9.09%

Supplementary Table 3.4 – Independent *t*-tests inheritance pattern in 22q11.2Dup

22q11.2Dup (*n* = 16)	***De novo* (*n* = 8)**	**Inherited (*n* = 8)**	**Statistical Outcomes Independent *t*-Test**
CCC-2 GCC			
Mean (*SD*)	104.25 (17.81)	101.63 (20.9)	$t = 0.277$
Median	101.00	105.50	$p = 0.786$
Range	78.00 – 129.00	76.00 – 135.00	$d = -0.138$
CCC-2 PC			
Mean (*SD*)	52.00 (9.90)	49.38 (11.70)	$t = 0.484$
Median	50.00	52.50	$p = 0.636$
Range	38.00 – 65.00	31.00 – 69.00	$d = -0.242$
SRS-2 SCI			
Mean (*SD*)	63.00 (15.54)	70.3 8 (27.22)	$t = -0.666$
Median	57.50	58.00	$p = 0.517$
Range	48.00 – 89.00	43.00 – 111.00	$d = 0.333$
SRS-2 RIB			
Mean (*SD*)	60.88 (14.02)	68.75 (27.98)	$U = 32.000$
Median	55.50	53.00	$p = 1.000$
Range	47.00 – 86.00	45.00 – 113.00	$r = 0.000$
SRS-2 Total			
Mean (*SD*)	63.13 (16.23)	71.25 (28.71)	$t = -0.697$
Median	56.00	57.50	$p = 0.497$
Range	49.00 – 91.00	44.00 – 114.00	$d = 0.348$

Note. Alpha = 0.05 *; alpha after Bonferroni correction = 0.01. **. GCC, General Communication Composite (norm group average = 80, cut-off: >104 = mild–moderate, ≥117 = severe); PC, Pragmatic Composite (norm group average = 40, cut-off: >53 = mild–moderate, ≥60 = severe); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour, Total (norm group average = 50, cut-off: >60 = mild–moderate, ≥76 severe).

Supplementary Table 3.5 – Independent *t*-tests sex in 22q11.2Dup

22q11.2Dup (*n* = 19)	**Female (*n* = 9)**	**Male (*n* = 10)**	**Statistical Outcomes Independent *t*-Test**
CCC-2 GCC			
Mean (*SD*)	101.89 (22.41)	101.30 (19.41)	$t = 0.061$
Median	103.00	102.00	$p = 0.952$
Range	72.00 – 135.00	76.00 – 129.00	$d = 0.460$
CCC-2 PC			
Mean (*SD*)	51.44 (9.72)	49.80 (11.16)	$t = 0.341$
Median	51.00	49.50	$p = 0.738$
Range	39.00 – 69.00	31.00 – 65.00	$d = 0.461$
SRS-2 SCI			
Mean (*SD*)	71.00 (24.98)	61.60 (14.30)	$t = 1.020$
Median	61.00	56.50	$p = 0.322$
Range	43.00 – 111.00	46.00 – 89.00	$d = 0.473$
SRS-2 RIB			
Mean (*SD*)	72.33 (23.74)	59.40 (14.37)	$t = 1.455$
Median	68.00	52.50	$p = 0.164$
Range	49.00 – 113.00	45.00 – 86.00	$d = 0.486$
SRS-2 Total			
Mean (*SD*)	72.44 (26.00)	61.70 (15.02)	$t = 1.118$
Median	63.00	57.00	$p = 0.279$
Range	44.00 – 114.00	46.00 – 91.00	$d = 0.475$

Note. Alpha = 0.05 *; alpha after Bonferroni correction = 0.01. **. GCC, General Communication Composite (norm group average = 80, cut-off: >104 = mild–moderate, ≥117 = severe); PC, Pragmatic Composite (norm group average = 40, cut-off: >53 = mild–moderate, ≥60 = severe); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour, Total (norm group average = 50, cut-off: >60 = mild–moderate, ≥76 severe).

Supplementary Table 3.6 – Independent *t*-tests country in 22q11.2Dup

22q11.2Dup (*n* = 16)	**Belgium (*n* = 10)**	**The Netherlands (*n* = 9)**	**Statistical Outcomes Independent *t*-Test**
CCC-2 GCC			
Mean (*SD*)	105.10 (15.48)	97.67 (24.98)	$t = 0.789$
Median	107.00	85.00	$p = 0.441$
Range	76.00 – 132.00	72.00 – 135.00	$d = 0.362$
CCC-2 PC			
Mean (*SD*)	50.80 (8.61)	50.33 (12.36)	$t = 0.096$
Median	52.50	45.00	$p = 0.924$
Range	31.00 – 62.00	38.00 – 69.00	$d = 0.044$
SRS-2 SCI			
Mean (*SD*)	66.20 (19.91)	65.89 (21.46)	$t = 0.033$
Median	60.00	57.00	$p = 0.974$
Range	46.00 – 107.00	43.00 – 111.00	$d = 0.015$
SRS-2 RIB			
Mean (*SD*)	64.70 (20.80)	66.44 (20.16)	$t = -0.185$
Median	55.50	68.00	$p = 0.855$
Range	45.00 – 113.00	47.00 – 107.00	$d = -0.085$
SRS-2 Total			
Mean (*SD*)	66.80 (21.01)	66.78 (22.39)	$t = 0.002$
Median	59.00	59.00	$p = 0.998$
Range	46.00 – 111.00	44.00 – 114.00	$d = 0.001$

Note. Alpha = 0.05 *; alpha after Bonferroni correction = 0.01. **. GCC, General Communication Composite (norm group average = 80, cut-off: >104 = mild–moderate, ≥117 = severe); PC, Pragmatic Composite (norm group average = 40, cut-off: >53 = mild–moderate, ≥60 = severe); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour, Total (norm group average = 50, cut-off: >60 = mild–moderate, ≥76 severe).

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Chapter 4

Chapter 4 - Language Profiles of School-Aged Children with 22q11.2 Copy Number Variants

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Abstract

Although it is known that copy number variants (CNVs) on chromosome 22, such as 22q11.2 deletion (22q11.2DS) and 22q11.2 duplication (22q11.2Dup) syndromes, are associated with higher risk for neurodevelopmental issues, few studies have examined the language skills across 22q11.2Dup nor compared them with the 22q11.2DS. The current study aims to characterise language abilities in school-aged children with 22q11.2Dup (n = 29), compared to age-matched children with 22q11.2DS (n = 29). Standardised language tests were administered, assessing receptive and expressive language skills across different language domains. Results indicate that children with 22q11.2Dup demonstrate significantly more language problems compared to the general population with large effect sizes. Mean language skills were not significantly different among children with 22q11.2 CNVs in this cohort. While children with 22q11.2DS demonstrated language difficulties starting at the word level, the most common language problems in children with 22q11.2Dup started at the sentence level. Importantly, both expressive and receptive language as well as lexico-semantic and morphosyntactic domains were impaired in children with 22q11.2 CNVs. Early identification, therapeutic intervention, and follow-up of language impairments in children with 22q11.2Dup are recommended to support language development and to reduce longitudinal impact of language and communicative deficits.

4.1 Introduction

Language and speech problems are major features of 22q11.2 deletion syndrome (22q11.2DS), with most children showing communication delays and up to 95% diagnosed with speech-language disorders (Solot et al., 2019; Van Den Heuvel, Manders, et al., 2018; Wenger et al., 2016). Since the duplication in the same chromosomal region is generally associated with milder phenotypes, one might wonder whether children with 22q11.2 duplication (22q11.2Dup) are less vulnerable to speech and language problems (Portnoï, 2009; Verbesselt, Zink, et al., 2022; Wenger et al., 2016).

Until now, little has been reported regarding language in children with 22q11.2Dup. Some case reports have mentioned speech or language delays but, in most instances, these problems were not further specified, and no clear distinction was made between speech and language problems (Cordovez et al., 2014; Courtens et al., 2008; Demily et al., 2018; Portnoï, 2009; Van Campenhout et al., 2012; Woodward et al., 2019; Yu et al., 2019). A prospective study using questionnaires compared social-communication skills in 19 children with 22q11.2Dup, 11 unaffected siblings, and 19 children with 22q11.2DS (Verbesselt, Van Den Heuvel, et al., 2022). Parents completed the Children's Communication Checklist (CCC-2), measuring speech, structural and pragmatic language, and social skills (Bishop, 2003, 2016). Speech-language delays were found in 79% (15/19) of children with 22q11.2Dup and 95% (18/19) of children with 22q11.2DS. Parents reported general communication problems in 47% of children with 22q11.2Dup (9/19), compared with 79% (15/19) of children with 22q11.2DS. The results also revealed that children with 22q11.2Dup were in an intermediate position between their siblings and children with 22q11.2DS (Verbesselt, Van Den Heuvel, et al., 2022). Another recent study (Verbesselt, Zink, et al., 2022) in 28 patients with 22q11.2Dup demonstrated delayed speech and language milestones in 68%. In addition, a subgroup of patients who underwent standardised testing showed language problems and one received a formal diagnosis of developmental language disorder (DLD). Longitudinal language data in six patients revealed a relatively stable trajectory in 3/6, catch-up with peers in 1/6, and a growing-into-deficit profile in 2/6. Growing into deficit means that patients are making insufficient progress with age, resulting in an increasing gap in language skills in relation to their typically developing peers (McDonald-McGinn et al., 2015; Swillen & McDonald-McGinn, 2015; Van Den Heuvel, Jonkers, et al., 2018; Verbesselt, Zink, et al., 2022).

Until now, language has only been evaluated in an indirect way through questionnaires or non-specific language testing. The current study aimed to characterise language profiles through direct standardised assessments in school-aged children with 22q11.2Dup and compare them to the skills of typically developing peers and age-matched children with the 22q11.2DS. To obtain a larger sample and higher statistical power, children seen at two different clinical genetics centres were studied: CME-Leuven in Belgium and Children's Hospital of Philadelphia (CHOP) in the USA. The following research questions are addressed.

1. Were both cohorts (Leuven and Philadelphia) sufficiently comparable to combine the data?

   If both cohorts were not statistically significantly different, their data would be combined for the subsequent analyses.

2. Were language skills of the two CNV groups (22q11.2Dup, 22q11.2DS) comparable to the scores of typically developing peers (norm group scores)?

   Language abilities of children in the CNV groups were expected to differ from the normative sample.

3. How did the language skills of children with 22q11.2Dup relate to those of age-matched children with 22q11.2DS?

   In accordance with previous indirect results (parent-reported), we hypothesised that children with 22q11.2Dup would fall between the general population and age-matched children with 22q11.2DS.

4. Did confounding factors have an impact on the language outcome, such as sex, comorbid ASD, ADHD, inheritance pattern, socioeconomic status (SES), and medical issues such as congenital heart disease (CHD), palatal defects, and hearing loss (HL)?

   Confounding factors were expected to have an impact on language outcomes in children with 22q11.2 CNVs.

5. Did genotype–phenotype correlations reveal duplicated regions or genes on 22q11.2 critical for language development?

   Genotype–phenotype correlations were expected to reveal critical regions on 22q11.2 for language development.

4.2 Materials and methods

4.2.1 Participants

A total of 58 school-aged children between 6 and 16 years of age were studied, consisting of 29 unrelated children with 22q11.2Dup and 29 unrelated children with 22q11.2DS. Exclusion criteria included: first language other than Dutch/English or <3 years of full-time Dutch/English education, extreme prematurity (i.e., gestational age <32 weeks), and moderate to severe hearing loss (≥35 dB HL) because of the known impact on language outcome (Barre et al., 2011; Crosbie et al., 2011; Cummins, 2000; De Houwer, 2021; Kohnert et al., 2021; Lieu et al., 2020). Additionally, children with CNVs outside of the standard LCR22A-LCR22D region or those children with more than one pathogenic chromosomal variant were excluded because of the lack of a minimally overlapping region and the impact on the phenotype, respectively. Children with comorbid neurodevelopmental disorders (NDDs) such as autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) were included, due to their high comorbidity with CNVs.

All participants with 22q11.2 CNVs had a laboratory confirmed diagnosis based on fluorescence in situ hybridisation (FISH), SNP microarray, or multiplex ligation-dependent probe amplification (MLPA). The typical 3 MB deletion/duplication with breakpoints situated at LCR22A-LCR22D was identified in all patients with 22q11.2DS (29) and 16 patients with 22q11.2Dup. Nested and larger duplications included LCR22A-LCR22B (2), LCR22A-LCR22E (2), LCR22A-LCR22H, LCR22B-LCR22C, LCR22B-LCR22F, and LCR22C-LCR22D (3). All duplications with breakpoints at the LCR22A-LCR22B region included the important developmental driver gene TBX1. More than half of the 22q11.2Dup were inherited (57%), while most 22q11.2DS occurred as a *de novo* event (95%). All 58 children with 22q11.2 CNVs were index patients referred for genetic testing due to medical/developmental/behavioural differences.

Table 4.1 includes the demographic and clinical characteristics of all participants. Children from CHOP were, on average, 1.9 years younger than children from the Leuven site. This difference was not statistically significantly different for 22q11.2Dup ($t = -1.776$, $p = 0.087$) or 22q11.2DS ($t = -1.740$, $p = 0.093$). Data on developmental milestones and education were retrieved from digital medical records/questionnaires completed by parents. Delayed speech-

language milestones were present in 67% of children with 22q11.2Dup and 96% of children with 22q11.2DS. Speech-language therapy was received by 83% of children with 22q11.2Dup and 100% of children with 22q11.2DS. Data on several genetic, environmental, developmental, and medical confounding factors were collected. Parental education (based on the mother's educational attainment) was used as a marker of SES and classified according to the International Standard Classification of Education (ISCED) of UNESCO (OECD, 2017; UNESCO Institute for Statistics., 2012) into three categories: low (primary education or lower grades of high school), middle (graduated from secondary/high school), high (graduated Bachelor, Masters, or Doctor of Philosophy). The presence and severity of congenital heart disease were classified by structural complexity using a three-point scale based on the classification by Billett et al. (2008), whereas palatal defects were classified as abnormal when having either a structural and/or functional palatal abnormality such as cleft lip/palate, cleft palate, submucous cleft palate or velopharyngeal dysfunction. Mild hearing loss was defined as having hearing thresholds between 20 and 35 dB HL (Michel, 2021).

Table 4.1 – Demographic and clinical features across groups

	22q11.2DS		**22q11.2Dup**	
Native Language (Country of Residence)	**Dutch (Belgium)**	**English (USA)**	**Dutch (Belgium)**	**English (USA)**
Sample Size (*n*)	18	11	18	11
Sex (*n*, %)				
Male	12 (67%)	8 (73%)	10 (56%)	7 (64%)
Female	6 (33%)	3 (27%)	8 (44%)	4 (36%)
Chronological age (yrs.mo)				
Average (*SD*)	10.10 (2.8)	9.1 (2.5)	10.10 (2.7)	9.1 (2.5)
Median	11.3	8.2	11.2	8.3
Range	6.7–15.2	6.4–13.2	6.11–15.5	6.4–13.3
Type of education (*n*, %)				
Special education	11 (61%)	2 (18%)	7 (39%)	3 (27%)
Regular education	6 (33%)	0 (0%)	6 (33%)	2 (18%)
Regular with assistance	1 (6%)	6 (55%)	5 (28%)	3 (27%)
Homeschool	0 (0%)	0 (0%)	0 (0%)	2 (18%)
Unknown	0 (0%)	3 (27%)	0 (0%)	1 (10%)
SES				
High	8 (44%)	5 (45%)	11 (61%)	2 (18%)
Middle	10 (56%)	5 (45%)	6 (33%)	4 (36%)
Low	0 (0%)	0 (0%)	1 (6%)	1 (10%)

Unknown	0 (0%)	1 (10%)	0 (0%)	4 (36%)
Speech-language delays (*n*, %)	18/18 (100%)	4/5 (80%)	14/18 (78%)	4/9 (44%)
Speech-language therapy (*n*, %)	18/18 (100%)	5/5 (100%)	15/18 (83%)	9/11 (82%)
Formal NDD diagnoses (*n*, %)				
ASD	7/18 (39%)	0/4 (0%)	2/18 (11%)	4/11 (36%)
ADHD	3/18 (17%)	0/4 (0%)	3/18 (17%)	3/10 (30%)
Inheritance pattern (*n*, %)				
De novo	17/18 (94%)	4/11 (36%)	8/18 (44%)	1/11 (10%)
Inherited:	1/18 (6%)	0/11 (0%)	7/18 (39%)	5/11 (54%)
Maternally inherited	1/1 (100%)	0/0 (0%)	2/7 (29%)	2/5 (40%)
Paternally inherited	0/1 (0%)	0/0 (0%)	5/7 (71%)	3/5 (60%)
Unknown	0/18 (0%)	7/11 (64%)	3/18 (17%)	5/11 (54%)
Medical issues (*n*, %)				
CHD	10/18 (56%)	7/11 (64%)	2/18 (11%)	1/11 (10%)
Palatal defects	11/18 (61%)	11/11 (100%)	4/18 (22%)	0/11 (0%)
Mild HL	9/18 (50%)	1/6 (17%)	2/18 (11%)	3/11 (27%)

Note. Abbreviations: SES, socioeconomic status; NDD, neurodevelopmental disorders; ASD, autism spectrum disorder; ADHD, attention-deficit/hyperactivity disorder; CHD, congenital heart defects; mild HL, hearing loss (≥ 20 dB HL and ≤ 40 dB HL).

4.2.2 Research design

Participants were recruited across two sites: the Centre for Human Genetics of UZ Leuven in Belgium and the 22q and You Centre at CHOP in the USA. For the CHOP cohort, data were obtained retrospectively through medical records/IRB approved REDCap database. Consequently, the amount of data available varied across different clinical features and patients, resulting in missing data for certain variables such as type of education, SES, speech-language delays, inheritance pattern and formal NDD diagnoses (Table 4.1). Hence, the total number of patients may vary depending on the available data in the described demographic and clinical features. For Dutch participants, data were prospectively collected during consultations at the hospital or home visits following a standardised research protocol. Further methodological details can be found in the study of Verbesselt et al. (2022), in which we have previously reported on a subgroup of these participants.

This study used a cross-sectional research design with both independent and pairwise comparisons. First, both cohort sites were compared across both CNV groups. Second, 29 chronological age-matched (CA) CNV pairs were compared. Cohort sites were considered by matching only within the same cohort. Age matching was within 0.7 years of age, with a mean difference of 1.72 months. Paired samples Wilcoxon signed-rank *t*-tests confirmed that there were no significant differences for the matching parameter ($W = 87.500$, $p = 0.617$, $r = 0.144$). According to Mervis and Klein-Tasman (2004), *p*-values of > 0.50 suggest that group distributions are sufficiently overlapping to be considered properly matched on the matching parameter (Mervis, 2004).

4.2.3 Measurements

Participants' language abilities were measured using standardised language instruments: the Dutch adaptation of the Clinical Evaluation of Language Fundamentals-Fourth edition (CELF-4- NL) (Kort et al., 2010) and/or the CELF-Preschool-Second Edition (CELF-P2-NL) (de Jong, 2012). The CELF-Third, Fourth and Fifth editions (CELF-3, CELF-4 and CELF-5) (Paslawski, 2005; Semel et al., 2003; Semel et al., 1995; Wiig et al., 2013) were used in the English-speaking cohort. The CELF assesses both receptive and expressive language across different language domains (i.e., semantics, syntax, and morphology); it is used in clinical practice to identify patients with language impairments, plan interventions, and evaluate progress over time. In addition, the CELF has normed references from 3–6 years of age on the CELF-P2 and 5–

18 (Dutch version) or 5–21 years of age (English version) on the CELF (versions 3–5). Based on the chronological age (CA) of the child, raw scores of each subtest were converted into scaled scores (SS) with a mean of 10 and a standard deviation (*SD*) of 3. Scaled scores of 7–13 were considered within the average range. Children with scaled scores of ≤6 were considered to have mild– moderate language problems, whereas scaled scores of ≤3 were interpreted as severe language problems. Receptive language subtests included the following: Concepts and Following Directions (CFD), Sentence Structure (SST), Understanding Spoken Paragraphs and Semantic Relationships (SR). Expressive language subtests included: Word Structure (WS), Recalling Sentences (RS), Formulated Sentences (FS), Word Classes (WC), Expressive Vocabulary (EV), Word Definitions (WD), and Sentence Assembly (SA). Core language, receptive, and expressive index scores were calculated based on CA (mean = 100, *SD* = 15) with a clinical cut-off of 85 (16th percentile, −1 *SD*) for mild language problems, 77 (6th percentile, −1.5 *SD*) for moderate language problems and 70 (2nd percentile, −2 *SD*) for severe language deficits.

The combination of receptive and expressive subtests to obtain the core, or composite, language scores (CLS) differed depending on the chronological age and test edition. In each test, however, the CLS was a measure for overall language ability. A review study found strong correlations between CELF-4 and CELF-5 composite scores (Coret & McCrimmon, 2015). Receptive language index (RLI) and expressive language index (ELI) were calculated to measure language production and comprehension. An additional expressive composite score was calculated based on the subtests Recalling Sentences (RS) and Formulated Sentences (FS), since these were consistently administered in children of all ages, regardless of the specific test or edition. The constructed composite was formed by the combined scaled scores of both subtests, with a mean scaled score of 20: e.g., a child with scaled scores of 6 on FS and 8 on RS has an expressive composite of 14.

4.2.4 Data analysis

Independent *t*-tests were used to investigate whether differences exist between the Leuven and CHOP cohort. Data were combined in the subsequent analyses once CNV groups (deletions – duplications) of both cohorts were determined to be comparable. Depending on the normality of the sample, Student's or Wilcoxon signed-rank one-sample *t*-tests were run to determine whether the language skills of the two target groups differ from the normative sample. Cross-CNV comparisons were carried out using paired sample Student's

t-tests. In addition, several genetic, environmental, developmental, and medical confounding factors were investigated through exploratory linear mixed models, with the CNV group and each confounding factor separately as fixed effects and CNV pairs as random effect.

Due to the anticipated heterogeneity within the target CNV groups, quantitative analyses were complemented with qualitative analyses and descriptive data were generated for all variables and groups. A p-value < 0.05 was considered statistically significant. Bonferroni correction was applied to reduce type I-errors because of multiple testing. Adapted p-values ranged from 0.008 to 0.025. For all outcome variables, 95% confidence intervals were calculated. Cohen's d was calculated as effect size measure for parametric analyses and values of 0.2, 0.5 and 0.8 were, according to Cohen's guidelines (Cohen, 1988; Ellis & Paul D, 2010), interpreted as small, moderate, and large effects, respectively. Analyses were carried out using JASP version 0.16.3 (JASP Team, 2022) and R 4.2.1 (R core team, 2017; Wickham, 2016).

4.3 Results

4.3.1 Cohort site differences

Figure 4.1 provides the boxplots of the CELF Core Language Scores (CLS) across cohort sites and CNV groups, with the light and darker grey zones delineating mild–moderate and severe language problems, respectively, and the dotted line referring to the mean in the normative sample. Mean composite scores across both cohort sites and CNV groups are summarised in Table 4.2. The table illustrates that, on average, children with 22q11.2Dup from CHOP had better language skills compared to the Dutch-speaking children, though independent *t*-tests revealed that the data were not statistically significantly different with small effect sizes, as shown in Table 4.2. In contrast, children with 22q11.2DS from both CHOP and Leuven showed similar language scores. The *p*-values of the 22q11.2DS group met the cut-off of $p > 0.50$ and, therefore, could be considered well matched, while the *p*-value for CLS in the 22q11.2Dup did not meet this criterion (Mervis & Klein-Tasman, 2004). Both cohort sites were not statistically significantly different but not properly matched either in the case of the duplications. Therefore, the subsequent analyses were conducted on the combined Leuven and CHOP cohort and on each cohort site separately.

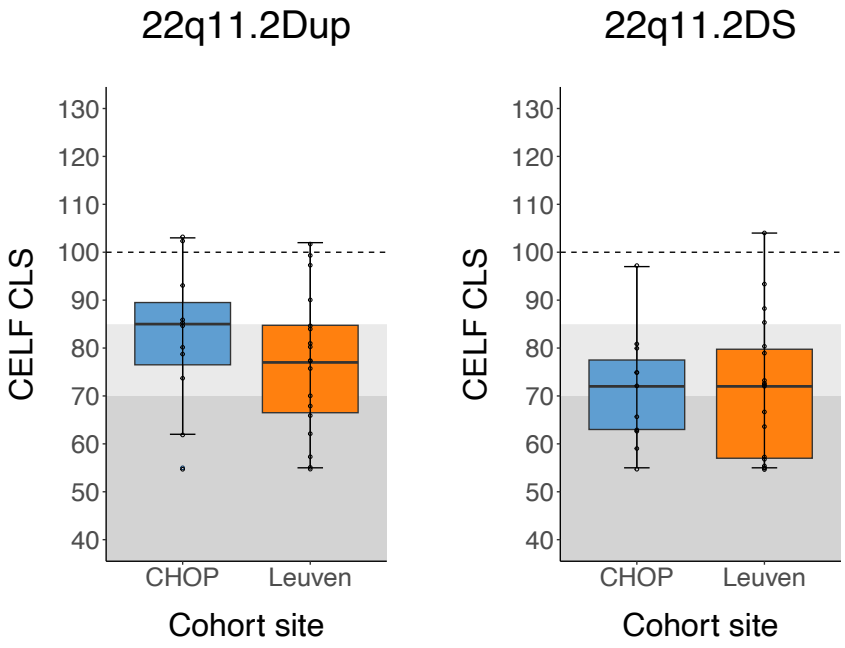

Figure 4.1 – Boxplots for CELF CLS composite scores across CNV groups and cohort sites.
The dotted lines show norm group averages. The grey zones indicate the severity of the problems; the darker the grey, the more severe the difficulties: mild–moderate = light grey zone and severe = darker grey zone, based on clinical cut-off scores for the CELF. Abbreviations. CELF, Clinical Evaluation of Language Fundamentals (norm group average = 100, cut-off: < 85 = mild–moderate (pc 16), < 70 = severe (pc 2).

Table 4.2 – Mean composite results on CELF across CNV groups and cohort sites.

	22q11.2Dup Dutch (*n* = 18)	**22q11.2Dup English (*n* = 11)**	**Statistical Outcomes Independent *t*-Test**	**22q11.2DS Dutch (*n* = 18)**	**22q11.2DS English (*n* = 11)**	**Statistical Outcomes Independent *t*-Test**
CELF CLS Mean (*SD*)	76.72 (14.67)	82.18 (14.84)	$t = 0.968$	71.33 (14.74)	71.46 (12.00)	$t = 0.023$
Range	55.00–102.00	55.00–103.00	$p = 0.342$	55.00–104.00	55.00–97.00	$p = 0.982$
95% Confidence interval	69.43–84.02	72.21–92.15	$d = 0.371$	64.00–78.66	63.39–79.52	$d = 0.009$
CELF RS + FS Mean (*SD*)	11.83 (6.19)	13.09 (5.89)	$t = 0.541$	10.28 (5.42)	11.46 (3.91)	$W = 107.000$
Range	2.00–24.00	4.00–22.00	$p = 0.593$	2.00–20.00	8.00–20.00	$p = 0.734$
95% Confidence interval	8.76–14.91	9.13–17.05	$d = 0.207$	7.58–12.97	8.83–14.08	$r = 0.081$

Note. Statistical outcomes: *p*-value; $\alpha = 0.05$; α after Bonferroni correction = 0.025; *t*-value or *W*-value. CELF, Clinical Evaluation of Language Fundamentals (norm group average = 100, cut-off: < 85: mild–moderate, < 70: severe); CLS, Core Language Score; RS, Recalling Sentences; FS, Formulated Sentences (norm group average = 20).

4.3.2 22q11.2 CNVs compared to norm group scores

Figure 4.2 displays the boxplots of the combined CELF CLS for each CNV group, with the dotted line pointing to the average of the normative sample and the grey zones showing the cut- offs for mild–moderate to severe core language impairments. A wide range of scores can be observed for both CNV groups.

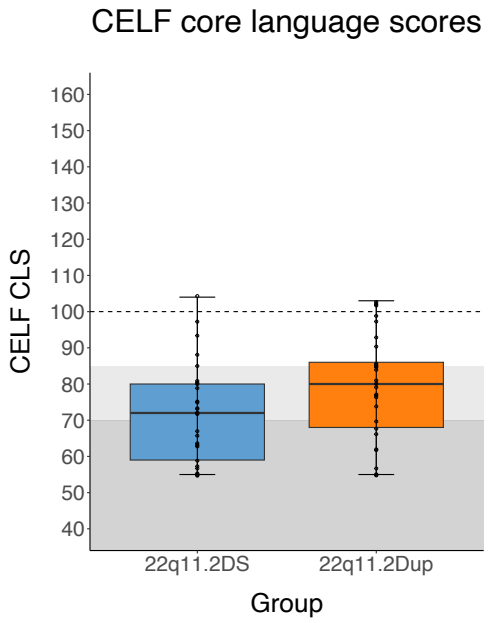

Figure 4.2 – Boxplots for CELF Core Language Scores across groups (22q11.2DS and 22q11.2Dup).

Comparative CELF core language scores are summarised in Figure 4.3. The dotted line represents the normal distribution of the normative sample (mean = 100, *SD* = 15). Compared to the norm group, negative shifts of 1.41 *SD* and 1.91 *SD* were observed in children with 22q11.2Dup and children with 22q11.2DS, respectively. The normative distributions of children with 22q11.2 CNVs show considerable overlap. One-sample Student's *t*-tests were carried out to determine whether the core language scores of the children with 22q11.2 CNVs differed from the normative sample. Results indicated that children with 22q11.2 CNVs scored statistically significantly lower on language compared to the norm group ($p < 0.001$) with large effect sizes ($d < -1.441$) and the results remained significant after Bonferroni correction.

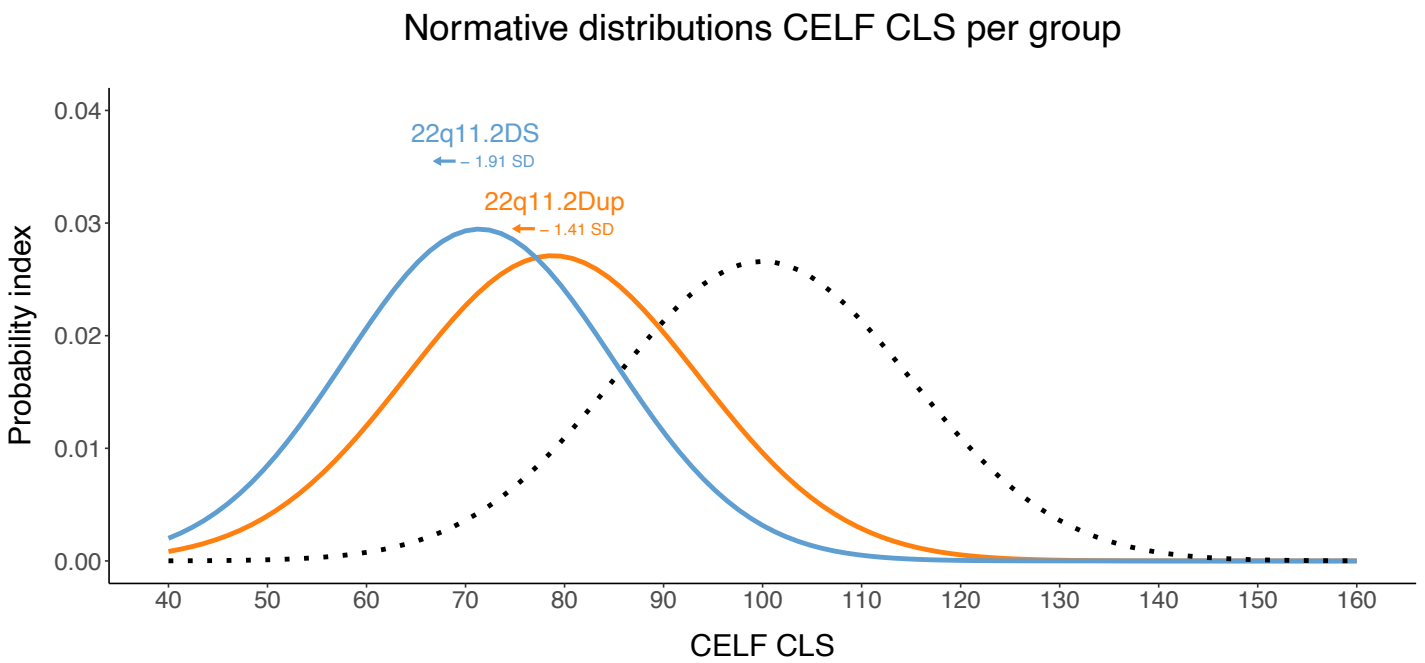

Figure 4.3 – Normative distributions of CELF Core Language Scores across groups (22q11.2DS and 22q11.2Dup).
SD, standard deviation. The dashed line illustrates the normative distribution of the norm group (mean = 100, *SD* = 15). *SD* shifts are calculated in relation to the normative sample.

4.3.3 Quantitative and qualitative cross-CNV comparisons

Mean CELF scores in Table 4.3 show mild–moderate core language impairments in children with 22q11.2Dup compared with moderate core language impairments in children with 22q11.2DS. Student's paired *t*-tests were performed to compare across CNVs, revealing no statistically significant differences in CLS between 22q11.2DS and 22q11.2Dup. Similarly, children with 22q11.2DS did not score significantly lower on the constructed expressive composite consisting of the subtests Recalling Sentences and Formulated Sentences. Similar scores were found for receptive (RLI) and expressive language indices (ELI) in children with 22q11.2Dup (mean RLI = 79.04, mean ELI = 77.00; $n = 27$) and in children with 22q11.2DS (mean RLI = 73.33, mean ELI = 71.67; $n = 12$). Subtest scores revealed similar distributions across all subtests, suggesting that children with 22q11.2DS and children with 22q11.2Dup in this sample have comparable language skills (Supplementary Table 4.1). Within the 22q11.2Dup group, children with delayed speech-language milestones in infancy ($n = 18$) demonstrated mean CLS of 73.44 compared to mean CLS of 88.67 in children without speech-language delays ($n = 9$). Independent *t*-tests confirmed that children with delayed milestones in infancy performed statistically significantly lower than children without speech-language delays ($t = -2.743$, $p = 0.011$, $d = -1.120$).

Table 4.3 – Cross-CNV comparisons across CELF CLS and expressive composite scores.

	Scores	22q11.2DS	22q11.2Dup	Statistical Outcomes Paired *t*-Test
CHOP + Leuven (*n* = 29)	CELF CLS Mean (*SD*)	71.38 (13.54)	78.79 (14.72)	*t* = 1.982
	Range	55.00–104.00	55.00–103.00	*p* = 0.057
	95% Confidence interval	66.23–76.53	73.19–84.39	*d* = 0.368
	CELF RS + FS Mean (*SD*)	10.72 (4.86)	12.31 (6.00)	*t* = 1.219
	Range	2.00–20.00	2.00–24.00	*p* = 0.233
	95% Confidence interval	8.87–12.57	10.03–14.59	*d* = 0.226
Leuven (*n* = 18)	CELF CLS Mean (*SD*)	71.33 (14.74)	76.72 (14.67)	*t* = 1.153
	Range	55.00–104.00	55.00–102.00	*p* = 0.265
	95% Confidence interval	64.00–78.66	69.43–84.02	*d* = 0.272
	CELF RS + FS Mean (*SD*)	10.28 (5.42)	11.83 (6.19)	*t* = 0.906
	Range	2.00–20.00	2.00–24.00	*p* = 0.378
	95% Confidence interval	7.58–12.97	8.76–14.91	*d* = 0.214
CHOP (*n* = 11)	CELF CLS Mean (*SD*)	71.46 (12.00)	82.18 (14.84)	*t* = 1.681
	Range	55.00–97.00	55.00–103.00	*p* = 0.124
	95% Confidence interval	63.39–79.52	72.21–92.15	*d* = 0.507
	CELF RS + FS Mean (*SD*)	11.46 (3.91)	13.09 (5.89)	*t* = 0.790
	Range	8.00–20.00	4.00–22.00	*p* = 0.448
	95% Confidence interval	8.83–14.08	9.13–17.05	*d* = 0.238

Note. Statistical outcomes: *p*-value; α = 0.05; α after Bonferroni correction = 0.008; *t*-value; Cohen's d as effect size. CLS, Core Language Score (norm group average = 100, cut-off: < 85: mild–moderate, < 70: severe); RS, Recalling Sentences; FS, Formulated Sentences (norm group average = 20).

Proportions of children with difficulties across CELF composite scores and subtests are summarised in Table 4.4. Based on the cut-off scores, core language impairments were ascertained in 62% of children with 22q11.2Dup and 83% of children with 22q11.2DS. There were severe impairments in 28% of children with 22q11.2Dup and 45% of children with 22q11.2DS. At the subtest level, the most common impairments in children with 22q11.2Dup were issues with Recalling Sentences in 66%, Concepts and Following Directions in 57%, Sentence Structure (5.0–8.11) or Semantic Relations (≥9.0) in 54% and Formulated sentences in 52%. In children with 22q11.2DS, the most common difficulties were problems with Word Definitions in 90%, Formulated Sentences in 76%, Concepts and Following Directions in 70%, Word Classes in 58%, Expressive Vocabulary in 57%, Recalling Sentences in 55%, and Sentence Comprehension (5.0– 8.11) or Semantic Relations (≥9.0) in 52%. Within the 22q11.2Dup group, most children with delayed speech-language milestones in infancy also showed impaired CLS (15/18), whereas 17% obtained average CLS (3/18). Most children without speech-language delays in infancy also showed average CLS (7/9), while 22% (2/7) showed impaired CLS. Within the 22q11.2DS group, most children with speech-language delays also demonstrated impaired CLS (18/22), whereas 18% (4/22) obtained average CLS. The child without speech-language delays also obtained average CLS.

Table 4.4 – Proportions of children with difficulties across composite and subtest scores on CELF.

		22q11.2DS	**22q11.2Dup**
Composite scores	CELF CLS (< −1 *SD* & < −2 *SD*)	24/29 (83%)	18/29 (62%)
	Mild–moderate < −1 *SD*	11/29 (38%)	10/29 (34%)
	Severe < −2 *SD*	13/29 (45%)	8/29 (28%)
	CELF RLI	10/12 (83%) *	19/27 (70%)
	CELF ELI	11/12 (92%)	18/28 (64%)
Subtest scores Receptive	CFD (5.00 – 12.11 years)	16/23 (70%)	12/21 (57%)
	SST / SR	14/27 (52%)	15/28 (54%)
	WC	11/19 (58%)	10/29 (34%)
Subtest scores Expressive	RS	16/29 (55%)	19/29 (66%)
	FS	22/29 (76%)	15/29 (52%)
	WS (5.0–8.11 years)	5/10 (50%)	6/12 (50%)
	EV (5.0–9.11 years)	4/7 (57%)	2/7 (28%)
	WD (≥ 10.00 years)	9/10 (90%)	6/12 (50%)

Note. * Available data vary by subtest due to different age ranges of specific subtests or missing data. CLS, Core Language Score; RLI, Receptive Language Index; ELI, Expressive Language Index (cut-off: < 85: mild–moderate, <70: severe); CFD, Concepts and Following Directions; RS, Recalling Sentences; FS, Formulated Sentences; WS, Word Structure; SST, Sentence Structure (5.0–8.11 years); SR, Semantic Relations (≥9.0 years); WC, Word Classes; EV, Expressive Vocabulary; WD, Word Definitions (cut-off: <7: mild–moderate problems; <4: severe problems).

4.3.4 Influence of confounding factors

Exploratory mixed models were carried out to delineate the impact of sex, comorbid ASD, ADHD, inheritance pattern, SES, and medical issues such as congenital heart disease (CHD), palatal defects, and mild hearing loss on language outcome, while accounting for intrafamilial pairs. Results indicate that none of the additional variables separately were statistically significantly associated with CELF CLS, except for high SES combined with specific CNV (CNV: $t = 2.511$, $p = 0.0154$; middle SES: $t = 1.988$, $p = 0.0524$; high SES: $t = 2.057$, $p = 0.0229$). The best fitted model included two factors in addition to the specific CNV ($t = 1.785$, $p = 0.0873$): the diagnosis of ASD ($t = -1.452$, $p = 0.1546$) and the SES (middle: $t = 1.593$, $p = 0.1190$; high: $t = 2.169$, $p = 0.0359$). Nevertheless, these associations were not statistically significant, except for high SES, although it did not remain significant after Bonferroni correction.

Descriptive data of scaled language scores were generated for all confounding factors. However, numbers were too imbalanced across groups to make meaningful comparisons and qualitative differences need to be interpreted with caution. Only qualitative differences on sufficiently large ($n = 5$) subgroups were explored. Within the 22q11.2Dup group, similar language scores were found independent of sex (average F: 76.4, M: 80.5), inheritance pattern (average *de novo*: 77.3, inherited: 79.7), and middle or high SES (average middle: 82.4, high: 80.4). Children with ASD ($n = 6$) performed, on average, five points higher than children without formal diagnosis of ASD ($n = 23$), while children with mild hearing loss ($n = 5$) scored, on average, nine points higher than children without hearing loss ($n = 24$).

Within the 22q11.2DS group, language scores were comparable regardless of sex (average F: 69.2, M: 73.4) and the presence of mild hearing loss (average HL: 73.5, no HL: 69.2). Children with either structural and/or functional palatal defects ($n = 22$) scored, on average, five points lower than children without palatal defects ($n = 7$). Children with ASD ($n = 7$) performed statistically significantly lower compared to children without formal diagnosis of ASD ($n = 15$, average ASD: 59.86, no ASD: 78.07, $n = 15$, $t = 4.089$, $p < 0.001$, $d = 1.668$). Finally, children with high SES ($n = 15$) scored, on average, eight points higher compared to children with middle SES ($n = 13$, average high: 75.4, middle: 67.3).

4.3.5 Genotype-phenotype correlations

Genotype–phenotype comparisons in typical duplications (LCR22A-LCR22D) versus nested/larger duplications distal to LCR22B reveal that 63% (10/16) children with the typical duplication show considerable language problems compared to 60% (3/5) children with nested/larger duplications without LCR22A-LCR22B. We delineated three minimal critical regions for language impairment: LCR22A-LCR22B in 13 children, LCR22B-LCR22C in 13 children, and LCR22C-LCR22D in 15 children.

4.4 Discussion

The current study aimed to characterise language skills using standardised language instruments in school-aged children with 22q11.2Dup, in comparison to skills of age-matched children with 22q11.2DS. Since children were studied from two cohort sites, cohort site-related differences were first explored. Results revealed no statistically significant differences between both cohorts for children with 22q11.2Dup or 22q11.2DS. On average, children with 22q11.2Dup from CHOP scored six points higher on CLS compared to children with 22q11.2Dup from Leuven. These results may be partially explained by the fact that children from CHOP were, on average, almost two years younger and that different versions of the same test (CELF-4 in Leuven vs. CELF-5 in CHOP) were used to assess language abilities. Cultural or spoken language differences between both countries may also contribute to these differences. Additionally, since children from CHOP were slightly younger, their normed scores may still decrease with increasing age, reflecting a growing-into-deficit profile, previously reported in a subgroup of the Leuven cohort and in children with 22q11.2DS (Solot et al., 2019; Verbesselt, Zink, et al., 2022). Finally, higher language outcomes in the CHOP cohort could be related to intellectual functioning in this group, an area requiring further study. Nevertheless, these rather small mean differences were not sufficient to consider groups as not comparable.

In agreement with the literature (Butensky et al., 2021; Chawner et al., 2021; Lin et al., 2020; Wenger et al., 2016), we found a slight male predominance in the current cohort of 22q11.2Dup. Percentages for medical issues fell within the range of reported percentages in previous both smaller and larger studies for CHD (0–24%) (Butensky et al., 2021; Clements et al., 2017; Dupont et al., 2015; Ensenauer et al., 2003; Portnoï, 2009; Wenger et al., 2016; Woodward et al., 2019; Zhang et al., 2021), mild hearing loss (4–42%) (Clements et al., 2017; Ensenauer

et al., 2003; Portnoï, 2009; Van Campenhout et al., 2012; Wenger et al., 2016; Woodward et al., 2019; Zhang et al., 2021), and palatal defects (8–25%) (Clements et al., 2017; Woodward et al., 2019; Yu et al., 2019). Other studies reported, on average, slightly higher rates of ASD (7–46%) and ADHD (27–44%) (Chawner et al., 2021; Lin et al., 2020; Olsen et al., 2018; Van Campenhout et al., 2012; Wenger et al., 2016; Woodward et al., 2019; Zhang et al., 2021) and lower rates of language delays (33–54%) (Woodward et al., 2019; Yu et al., 2019; Zhang et al., 2021). The current cohort of 22q11.2DS consisted of a relatively high proportion of males, whereas other larger studies reported more even male/female distributions. In addition, the current cohort demonstrated high rates of medical issues (CHD and palatal defects) and elevated rates of NDD (ASD and ADHD), which is in line with other studies (Biswas & Furniss, 2016; Campbell et al., 2018; Chawner et al., 2021; Lin et al., 2020; McDonald-McGinn et al., 2015; Wenger et al., 2016). Based on these findings, the current cohort of children with 22q11.2 CNVs seemed to be representative of what has been described in the literature so far.

Comparisons to the normative sample confirmed that children with 22q11.2 CNVs have statistically significantly lower language scores in relation to typically developing peers, which is in accordance with results based on parental reports (Verbesselt, Van Den Heuvel, et al., 2022). In addition, a shift of approximately −2 *SD* in the 22q11.2DS group is consistent with findings on their cognitive profiles in previous studies (Chow et al., 2006; De Smedt et al., 2007; Fiksinski et al., 2022; Olszewski et al., 2014). We found a shift of approximately −1.5 *SD* in the 22q11.2Dup group, which is at the lower end of the range of their cognitive capabilities based on previous studies. In particular, Chawner et al. (2021) found a downward shift of 0.8 *SD* with a mean FSIQ of 88 in 32 patients with 22q11.2Dup, whereas Modenato et al. (Modenato, Kumar, et al., 2021; Modenato, Martin-Brevet, et al., 2021) reported mean FSIQ of 97.82 in 12 patients and mean downward shift of 1.51 in 44 patients. Similarly, Verbesselt et al. (2022) found mean FSIQ of 76 in 19 patients, corresponding to a downward shift of 1.6 *SD*. Although 22q11.2 CNVs appeared to shift the distribution to the left compared to the general population, they did not change its clinical features.

While there was an average seven-point difference (0.5 *SD*) in core language scores favouring the children with 22q11.2Dup, the differences were not statistically significant. Differences were expected because duplications are generally associated with milder phenotypes compared to deletions (Goldenberg, 2018). In addition, parents reported more communication problems in children

with 22q11.2DS than in children with 22q11.2Dup (Verbesselt, Van Den Heuvel, et al., 2022). General communication concerns were reported in 47% of children with 22q11.2Dup and 79% of children with 22q11.2DS, whereas the current study found considerable language problems in 62% of children with 22q11.2Dup compared with 83% of children with 22q11.2DS. Therefore, results based on parental reports might reflect that parents of children with 22q11.2Dup may underestimate the language difficulties of their children. One might ask whether this finding is related to the fact that duplications are more often inherited and that affected parents might experience developmental issues and hence have difficulties with completing the questionnaires and/or correctly assessing the abilities of their children (Pizzo et al., 2019). Nevertheless, many parents of children with the 22q11.2Dup were highly educated in the current study. More likely, discrepancies might be related to the subjective nature of indirect assessment methods. Therefore, indirect measurements such as questionnaires should be validated by direct assessment such as standardised language instruments to provide additional information about the true language capacities of the child. Another possible explanation for this discrepancy might be the fact that the CELF assesses structural and semantic language components, whereas the CCC-2 questionnaire screens speech, structural, semantic, and pragmatic language skills. Consequently, the lower reported proportion of communicative problems (47%) may be attributed to better speech or better pragmatic than structural and semantic language skills in children with 22q11.2Dup.

Mean language scores of both CNV groups could be classified as within the range of mild– moderate language impairments. Within both CNV groups, similar scores were found for RLI and ELI. The presence of differences between receptive and expressive language in 22q11.2DS is the subject of debate in the literature. Pre-school children with 22q11.2DS often showed higher receptive than expressive skills, while there was more varied reporting in the literature on receptive–expressive discrepancies in the school-aged population (Gerdes et al., 2001; Glaser et al., 2002; Persson et al., 2006; Solot et al., 2001; Van Den Heuvel, Manders, et al., 2018). The current results in the 22q11.2Dup group suggest that children with 22q11.2Dup might experience comparable receptive and expressive language challenges. Future longitudinal studies should clarify whether these similar receptive and expressive deficits are characteristic of the 22q11.2Dup population. Within the 22q11.2Dup group, children with speech-language delays in infancy obtained statistically significantly lower core language scores at primary school age than children without speech-language delays. These

results might suggest that delayed milestones in infancy are indicative of language impairments in primary school. Nevertheless, these results should be interpreted with caution, since a history of speech-language delays did not always lead to core language impairments (n = 3, 17%) and others still developed language impairments without a history of speech-language delays (n = 2, 22%).

Children with 22q11.2DS showed higher proportions of difficulties with subtests at the word level, such as Word definitions, Expressive Vocabulary, and Word Classes, whereas children with 22q11.2Dup predominantly demonstrated difficulties with subtests on the sentence level. However, difficulties with word-level subtests were not equally representative due to the smaller age range and accompanying smaller sample. Regarding the remaining subtests, the most common problems were found across the same subtests for children with 22q11.2 CNVs, but higher proportions of difficulties were found in the 22q11.2DS group. Therefore, children with 22q11.2 CNVs seemed to have deficits across several language domains, including both lexico-semantic problems (based on Concepts and Following Directions, Sentence Structure/Semantic Relations) and morpho-syntactic problems (based on Recalling Sentences and Formulated Sentences). Moreover, both language production and comprehension may be impaired in both CNV groups. Results for the expressive language composite based on Recalling Sentences and Formulated Sentences were in line with the core language scores. Higher proportions of difficulties with Concepts and Following Directions and Recalling Sentences might be related to impaired working memory, attention, and executive functioning in both groups of children, which has been previously described in 22q11.2DS (Antshel et al., 2017; Maeder et al., 2016, 2022; Moberg et al., 2018; Montojo et al., 2014; Swillen et al., 2018; Van Den Heuvel, Manders, et al., 2018). Thus, qualitatively, children with 22q11.2Dup seemed to show lower proportions of language difficulties and higher language scores, with challenges across similar domains compared to children with 22q11.2DS.

Regarding confounding factors, only comorbid ASD, SES, and the specific CNV seemed to play a role in the language scores, although these effects did not remain statistically significant after correction for multiple testing. Children with ASD only performed worse in the 22q11.2DS group and children with high SES only showed higher scores in the 22q11.2DS group. Consequently, these factors did not seem to influence language outcomes in children with 22q11.2Dup. Qualitatively, the opposite pattern was shown for other variables, such as the observation of higher language scores in children with 22q11.2Dup

and mild hearing loss or ASD. However, these differences cannot be generalised and should be interpreted with caution due to the small and imbalanced numbers. Moreover, differences for hearing loss and ASD were smaller than one standard deviation, and, thus, clinically less relevant. Future studies with more participants are needed to further delineate the impact of these and other potentially confounding factors. To conclude, qualitatively, children with 22q11.2Dup seemed to be in an intermediate position between the general population and age-matched children with 22q11.2DS, albeit with more overlap with the 22q11.2DS group.

Language problems were observed both in patients with duplications proximal to LCR22B, as well as in patients with more distal duplications, pointing toward downstream effects or the presence of multiple copy number sensitive loci for language development on chromosome 22q11. Minimal regions of overlap in patients with language problems included LCR22A-LCR22B, LCR22B-LCR22C and LCR22C-LCR22D. Woodward et al. (2019) described thirteen atypical, nested duplications and investigated candidate genes within the LCR22B-LCR22D interval that might be associated with brain development and ASD traits. Based on gene expression in tissue of the nervous system, nine genes (ZNF74, KLHL22, MED15, PI4KA, SERPIND1, CRKL, AIFM3, SLC7A4, and BCRP) in the LCR22B-LCR22D interval were selected as candidate genes for these traits. Their potential link to language impairment in particular has not been established so far. Future studies with larger samples of typical and nested 22q11.2Dup, in addition to case-control variant burden studies in extensive cohorts of patients with severe language problems, are required to study the contribution of variation in genes on 22q11.2 to abnormal language development.

4.4.1 Strengths, limitations and future

To the best of our knowledge, this is the first study to measure language in children with 22q11.2Dup. By combining data from two cohort sites, a relatively large sample was obtained, increasing statistical power. However, data in CHOP were retrospectively collected, resulting in missing data for certain variables, such as information on inheritance pattern, SES, type of education, and the presence of NDD or mild hearing loss. The current sample size, although relatively large for these CNVs, might still prevent us from finding significant differences between 22q11.2 CNVs. Hence, future studies with larger samples are needed to confirm the current results. Another methodological limitation is the use of different versions of the same language test, which might lead to

slightly different outcomes. Nevertheless, all CELF editions are constructed to measure the same language components and overall language ability and are recognised and used in clinical practice. Additionally, the self-constructed expressive composite showed similar results compared to the CLS, confirming its reliability and validity. Other strengths of the current study include the use of gold standard language assessments, strict inclusion and exclusion criteria, and comparisons with the normative sample. The large variability within the CNV groups points to the role played by other factors in language outcomes, such as the broader genetic background, IQ, hearing, ASD, ADHD, SES, which should be further elucidated in future studies.

As the present study only characterised semantic and structural language skills, future studies should also delineate pragmatic skills and speech abilities through direct assessments, just as was established in 22q11.2DS (Solot et al., 2019; Van Den Heuvel et al., 2017). All patients were diagnosed based on medical or developmental indications for genetic testing and only probands were included. Consequently, language outcomes may reflect the more severe end of the phenotypic spectrum due to ascertainment bias in this clinical cohort. It is likely that milder language impairments are present in those individuals identified with the 22q11.2Dup only following the diagnosis in their affected relative (e.g., a parent or sibling) as they may not have come to attention a priori with medical or developmental problems. Future studies should also include non-probands and affected relatives to obtain a complete picture of language skills in the 22q11.2Dup population. In addition, language should be interpreted in relation to the overall cognitive profile given the relationship between language and cognition. Hence, it would be an added value to perform intelligence testing prospectively in future research. Finally, the inclusion of larger samples and the collection of longitudinal data may lead to a more complete overview of the phenotypic spectrum of children with 22q11.2Dup across the lifespan.

4.5 Conclusions

This is the first study to characterise language skills in children with 22q11.2Dup through direct language instruments, in relation to typically developing peers in the general population and age-matched children with 22q11.2DS. Considerable language difficulties were found in a high proportion of children with 22q11.2 CNVs. Therefore, as in 22q11.2DS, regular follow-up of language development in children with 22q11.2Dup is advised. Early screening and characterisation of language skills in 22q11.2Dup are recommended to identify children who qualify for educational support in school or speech-language therapy through a rehabilitation centre or private practice. Finally, as in 22q11.2DS, adapted treatment is suggested to support and improve language skills and to reduce potential long-term influence of language and communicative deficits (Solot et al., 2019).

4.6 Supplementary material

Supplementary Table 4.1 – Subtest scores across children with 22q11.2 CNVs

			22q11.2DS	22q11.2Dup
Summary scores	RLI			
		N	12	27
		M (SD)	73.33 (10.11)	79.04 (14.14)
		Median	76.00	81.00
		Range	59.00 – 91.00	55.00 – 109.00
	ELI			
		N	12	28
		M (SD)	71.67 (13.14)	77.00 (15.50)
		Median	70.00	81.00
		Range	55.00 – 104.00	55.00 – 104.00
Subtest scores receptive	CFD (5.00 – 12.11 years)			
		N	23	21
		M (SD)	5.22 (2.97)	5.71 (2.90)
		Median	5.00	5.00
		Range	1.00 – 13.00	2.00 – 11.00
	SST / SR			
		N	27	28
		M (SD)	6.30 (3.29)	6.21 (3.28)
		Median	6.00	6.00
		Range	1.00 – 14.00	1.00 – 13.00
	SST (5.00 – 8.11 years)			
		N	12	12
		M (SD)	5.67 (2.93)	6.83 (3.41)
		Median	5.50	7.00
		Range	1.00 – 10.00	2.00 – 13.00
	SR (≥9.00 years)			
		N	15	16
		M (SD)	6.80 (3.57)	5.75 (3.22)
		Median	7.00	6.00
		Range	1.00 – 14.00	1.00 – 11.00
	WC			
		N	19	29
		M (SD)	5.79 (2.76)	7.28 (2.87)
		Median	6.00	7.00
		Range	1.00 – 10.00	2.00 – 13.00
Subtest scores expressive	RS			
		N	29	29
		M (SD)	6.21 (3.01)	5.97 (3.09)
		Median	6.00	6.00
		Range	1.00 – 13.00	1.00 – 13.00
	FS			
		N	29	29
		M (SD)	4.52 (2.32)	6.35 (3.50)

Median	4.00	6.00
Range	1.00 – 9.00	1.00 – 14.00
WS (5.00 – 8.11 years)		
N	10	12
M (SD)	7.20 (2.44)	6.25 (2.45)
Median	7.00	6.50
Range	4.00 – 12.00	3.00 – 10.00
EV (5.00 – 9.11 years)		
N	7	7
M (SD)	5.86 (3.63)	7.57 (3.65)
Median	6.00	7.00
Range	1.00 – 10.00	3.00 – 14.00
WD (≥10.00 years)		
N	10	12
M (SD)	3.60 (2.32)	5.83 (2.55)
Median	3.00	6.50
Range	1.00 – 8.00	1.00 – 9.00

Note. * Available data vary by subtest due to different age ranges of specific subtests or missing data. Abbreviations: CFD, Concepts and Following Directions (5.00 – 12.11 years); RS, Recalling Sentences; FS, Formulated Sentences; WS, Word Structure; SST, Sentence Structure (5.00 – 8.11 years); SR, Semantic Relations (≥9.00 years); WC, Word Classes; EV, Expressive Vocabulary; WD, Word Definitions (cut-off: <7: mild–moderate problems; <4: severe problems).

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Chapter 5

Chapter 5 - Association of behavioural and social-communicative skills in school-aged children with 16p11.2 copy number variants: a multi-site study

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Abstract

Background: Despite the established knowledge that recurrent copy number variants (CNVs) at the 16p11.2 locus BP4-BP5 confer risk for behavioural and language difficulties, limited research has been conducted on the association between behavioural and social-communicative profiles. The current study aims to further delineate the prevalence, nature and severity of, and the association between behavioural and social-communicative features of school-aged children with 16p11.2 deletion syndrome (16p11.2DS) and 16p11.2 duplication (16p11.2Dup).

Methods: A total of 68 individuals (n = 47 16p11.2DS, n = 21 16p11.2Dup) aged 6-17 years participated. Standardised intelligence tests were administered, and behavioural and social-communicative skills were assessed by standardised questionnaires. Scores of both groups were compared to population norms and across CNVs. The influence of confounding factors was investigated and correlation analyses were performed.

Results: Compared to the normative sample, children with 16p11.2DS showed high rates of social responsiveness and communicative problems (69%), while approximately half (52%) of the patients displayed behavioural problems. Children with 16p11.2Dup demonstrated even higher rates of social-communicative problems (73-90%) with statistically significantly more externalising and overall behavioural challenges (89%). In both CNV groups,

there was a strong positive correlation between behavioural and social-communicative skills.

Conclusion: School-aged children with 16p11.2 CNVs show high rates of behavioural, social- responsiveness and communicative problems compared to the normative sample. These findings point to the high prevalence of autistic traits and diagnoses in these CNV populations. Moreover, there is a high comorbidity between behavioural and social-communicative problems. Patients with difficulties in both domains are vulnerable and need closer clinical follow-up and care.

5.1 Introduction

Copy number variants (CNVs) at the 16p11.2 locus, such as 16p11.2 deletion syndrome (16p11.2DS) and 16p11.2 duplication (16p11.2Dup) between breakpoints 4 and 5 (BP4-BP5), involving approximately 593 kb and 29 genes, confer susceptibility to neurodevelopmental difficulties (Deshpande & Weiss, 2018; Zarrei et al., 2019). The 16p11.2DS occurs *de novo* in approximately 71% of cases, while the 16p11.2Dup is mostly inherited (70%) (D'Angelo et al., 2016; Niarchou et al., 2019). Both CNVs are characterised by both contrasting and overlapping features across medical and neurodevelopmental domains. The medical phenotype usually implicates underweight in 16p11.2Dup, overweight/obesity in 16p11.2DS and epilepsy in both, whereas neurodevelopmental features overlap and include learning capacities ranging from intellectual disability (ID) to average IQ, motor problems, speech/language impairments and behavioural difficulties (Deshpande & Weiss, 2018; Knoll et al., 2018; Rein & Yan, 2020; Taylor et al., 2021).

Despite the high comorbidity between neurodevelopmental disorders (NDDs) in 16p11.2 CNVs (Green Snyder et al., 2016; Hanson et al., 2015), a dimensional approach linking the underlying skills across these domains remains elusive. To describe behavioural features on a continuous scale, some studies used the Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2001; Verhulst et al., 2001) and Social Responsiveness scale (SRS-2; Constantino & Gruber, 2012; Roeyers et al., 2015), predominantly finding borderline CBCL scores and severe SRS-2 scores in both CNV groups, confirming the presence of mild to moderate behavioural problems and severe autistic traits (Bernier et al., 2017; Green Snyder et al., 2016; Hanson et al., 2015; Moreno-De-Luca et al., 2015; Smith et al., 2022; Taylor et al., 2023). However, comparative analyses of these social and behavioural skills across school-aged children with 16p11.2

CNVs are lacking. In addition, detailed characterisation of behavioural (CBCL) and social responsiveness (SRS-2) subdomains is limited.

Using the Children's communication checklist (CCC-2; Bishop, 2003; H. Geurts, 2007), Kim et al. (2020) ascertained high rates of both syntactic and pragmatic-semantic delays in children aged 4-16 years with 16p11.2 CNVs, with significantly more syntactic problems in deletion carriers. They concluded that language impairments persist after controlling for autism spectrum disorder (ASD) and ID. However, little is known about the relation between communicative impairments (CCC-2), autistic traits (SRS-2), behavioural problems (CBCL) and cognitive functioning in these CNV populations. In addition, the influence of inheritance pattern and socioeconomic status on these skills has not been characterised before. Unlike previous research predominantly focused on the Simons Searchlight (The Simons VIP Consortium, 2012), our study also incorporates findings from a European cohort.

This multi-site study has three general objectives: The first aim is to characterise the presence, nature and severity of behavioural and social-communicative skills in school-aged children (6-17years) with 16p11.2 CNVs, compared to the normative sample and across CNVs. We aim to provide a detailed analysis by considering both summary and subtest scores. Cross-CNV comparisons may reveal syndrome-specific features (Mervis, 2004) and highlight gene dosage-linked profiles across behavioural and social-communicative domains, since mirror phenotypes were reported before for head circumference and BMI at the 16p11.2 locus (Jacquemont et al., 2011). Secondly, the influence of confounding factors, such as age, IQ, sex, inheritance pattern, socioeconomic status (SES) and cohort site will be explored for each CNV. Thirdly, we want to investigate whether associations exist between behavioural, social responsiveness, and communicative skills and their relationship with cognitive functioning in both CNV groups. This aspect of the study holds particular importance as it has the potential to uncover crucial insights into the interplay between cognitive abilities and social-behavioural phenotypes in individuals with CNVs.

5.2 Materials and methods

5.2.1 Participants

A total of 68 school-aged participants were enrolled in this study (*M* = 10y4m, *SD* = 3y1m, range 6y1m – 17y1m). All children were unrelated index patients who received the genetic diagnosis of 16p11.2DS or 16p11.2Dup through microarray (array CGH) or exome sequencing with CNV calling. Cases with an additional CNV were reviewed by an expert clinical geneticist (J.B.) and excluded if the CNV was classified as (likely) pathogenic. Other exclusion criteria included: extreme prematurity (i.e., gestational age < 32 weeks), moderate/severe hearing loss (≥ 35 dB HL), and native language other than Dutch/English or < 3 years of full-time Dutch/English education (Barre et al., 2011; Crosbie et al., 2011; Cummins, 2000; Kohnert et al., 2021; Lieu et al., 2020). Table 5.1 shows the sociodemographic characteristics for both CNVs across the two cohorts. The total number of patients varied across different clinical features and several sociodemographic characteristics, resulting in missing data.

Table 5.1 – Sociodemographic characteristics across cohort sites and CNV groups

	16p11.2DS		**16p11.2Dup**	
Cohort – Native language	**Leuven – Dutch**	**Geisinger – English**	**Leuven – Dutch**	**Geisinger – English**
Sample Size (*n*)	24	23	11	10
Sex (*n*, %)				
Male	10 (42%)	12 (52%)	5 (45%)	4 (40%)
Female	14 (58%)	11 (48%)	6 (55%)	6 (60%)
Chronological age (yrs.mo)				
Average (*SD*)	10.11 (3.2)	12.0 (3.2)	10.5 (2.11)	12.3 (3.3)
Median	10.2	12.5	9.7	13.5
Range	6.7 – 16.11	6.2 – 17.1	6.1 – 14.7	6.1 – 16.7
Type of education (*n*, %)				
Special education	20 (83%)	0 (0%)	9 (82%)	4 (40%)
Regular education	3 (13%)	0 (0%)	2 (18%)	0 (0%)
Regular with assistance	1 (4%)	1 (4%)	0 (0%)	1 (10%)
Unknown	0 (0%)	22 (96%)	0 (0%)	5 (50%)
SES*				
High	8 (34%)	6 (26%)	4 (36%)	2 (20%)
Middle	12 (50%)	4 (17%)	4 (36%)	2 (20%)
Low	1 (4%)	0 (0%)	3 (28%)	1 (10%)
Unknown	2 (8%)	13 (57%)	0 (0%)	5 (50%)
Speech-language delays (*n*, %)	22/24 (92%)	1/1 (100%)	11/11 (100%)	5/5 (100%)

Speech-language therapy (*n*, %)	19/24 (79%)	1/1 (100%)	11/11 (100%)	5/5 (100%)
Formal NDD diagnoses (*n*, %)**				
ID (FSIQ < 70)	10/23 (43%)	4/13 (31%)	7/11 (64%)	2/6 (33%)
ASD	11/24 (46%)	6/21 (29%)	7/11 (64%)	4/9 (44%)
ADHD	7/24 (29%)	2/12 (17%)	6/11 (55%)	5/5 (100%)
Inheritance pattern (*n*, %)				
De novo	13/24 (54%)	13/23 (56%)	3/11 (27%)	0/10 (0%)
Inherited:	5/24 (21%)	5/23 (22%)	2/11 (18%)	5/10 (50%)
Maternally inherited	3/5 (60%)	3/5 (60%)	2/2 (100%)	3/5 (60%)
Paternally inherited	2/5 (40%)	2/5 (40%)	0/2 (0%)	2/5 (40%)
Unknown***	6/24 (25%)	5 /23(22%)	6/11 (55%)	5/10 (50%)

Note. Abbreviations: SES, socioeconomic status; NDD, neurodevelopmental disorders; ID, intellectual disability; ASD, autism spectrum disorder; ADHD, attention-deficit/hyperactivity disorder. *Educational attainment of the mother was used as a proxy for socioeconomic status (SES). The classification of SES was based on the International Standard Classification of Education (ISCED) of UNESCO (OECD, 2017; UNESCO Institute for Statistics., 2012), using three categories: low (primary education or lower grades of high school), middle (secondary/high school graduate), high (Bachelor, Master's, or Doctoral Degree). **All formal NDDs were diagnosed by a multidisciplinary team, using gold standard diagnostic instruments such as Autism Diagnostic Observation Schedule – Second Edition (ADOS-2) and Autism Diagnostic Interview – Revised (ADI-R) for ASD, and psychiatric interviews, according to Diagnostic and Statistical Manual of Mental Disorders, fifth version (DSM-5) criteria (American Psychiatric Association, 2013; de Bildt et al., 2013; de Jonge et al., 2003; Lord et al., 2012; Rutter et al., 2003). ***adopted (*n* = 7), foster care (*n* = 2), maternal inheritance ruled out, parents declined genetic testing …

5.2.2 Procedures and measures

Leuven participants were recruited through the Centre for Human Genetics at University Hospitals Leuven. The Geisinger cohort consisted predominantly of patients recruited through the Autism & Developmental Medicine Institute Geisinger, supplemented by patients recruited through the University of Washington and Washington University in St. Louis. A subset of patients in the Geisinger cohort (16/23 16p11.2DS,4/10 16p11.2Dup) were also enrolled in Simons Searchlight. Patients from both sites were referred to the genetics or developmental medicine clinic either due to medical indications, neurodevelopmental concerns, or a combination of both. To avoid bias caused by intrafamilial associations, CNV-carrying siblings of probands were excluded. Data were prospectively collected during home visits or consultations at the hospital from 2015 to 2023, following a standardised research protocol.The research protocol consisted of a standardised IQ-test, and three standardised questionnaires, completed by parents: the Child Behavior Checklist 6-18 (CBCL; Achenbach & Rescorla, 2001; Verhulst et al., 2001), the Children's Communication Checklist – Second Edition (CCC- 2; Bishop, 2003; H. Geurts, 2007) and the Social Responsiveness Scale – Second Edition (SRS-2; Constantino & Gruber, 2012; Roeyers et al., 2015). Data on sociodemographic characteristics, NDD diagnoses, language milestones and provision of therapy were based on clinical files and caregiver reports, collected through anamnestic interviews and questionnaires.

Intelligence test (IQ test)

An age-appropriate intelligence test was administered in all Leuven and most Geisinger participants (Supplementary Table 5.1). Full-scale IQ (FSIQ) was calculated ($M = 100$, $SD = 15$) for each participant. Since one participant was not verbal at the age of 10, only a non-verbal IQ could be calculated.

Child Behavior Checklist (CBCL)

The CBCL 6-18 is a screening instrument in the evaluation of emotional and behavioural problems, identifying difficulties on eight syndrome subscales and yielding three summary scores (Externalising, Internalising and Total Problems). Raw scores for each scale are converted to standard *T*-scores ($M = 50$, $SD = 10$), using sex- and country-dependent norms for school-aged children between 6-18 years of age. Cut-offs for clinically elevated scores are indicated by *T*-scores ≥ 70 (pc <3) on the syndrome subscales and ≥ 64 on the summary scores

(pc 8). Cut-offs for borderline elevated scores range from 65-69 (pc 3-7) and 60-63 (pc 10-16) for the syndrome subscales and summary scores, respectively (Achenbach & Rescorla, 2001; Verhulst et al., 2001).

Children's Communication Checklist (CCC-2)

The CCC-2 is a 70-item questionnaire tapping into communicative behaviours across ten different subscales. Using age-appropriate norms, raw scores are converted into scaled scores (SS) for each subscale (M = 10, SD = 3). The American version is normed for children between 4-16 years, while the Dutch version is normed between 4-15.6 years. Since three children were already older than the normed maximum age, their scores were converted using the norm table of the eldest available norm group.

In the Dutch version, higher scores are interpreted as weaker communicative skills, with scores of >13 (= M + 1 SD) and >16 (= M + 2 SD) as cut-offs for mild-moderate and severe communicative deficits, respectively. Since the scores of the American version are interpreted inversely (the higher the score, the better the communicative skills), scores for Geisinger participants were transformed to be compatible with the Leuven data. If a child obtains a SS of 17, the parents have severe concerns about their child's communicative skills. Two summary scores can be calculated; the General Commmunication Composite (GCC), based on the sum of scaled scores of the first eight subscales, and the Pragmatic Composite (PC), based on the sum of the four pragmatic subscales. In the American version, the raw sum of scaled scores is then scaled (M = 100, SD = 15). However, as the Dutch version has no conversion tables to report scaled values, we only used the raw sum of scaled scores for both cohort sites for statistical analyses, with >104 (pc 10) and ≥117 (pc 2) as cut-off scores for moderate or severe communicative problems for the GCC (M = 80); and similarly, with >53 (pc 10) and ≥60 (pc 2) as cut-off scores for the PC (M = 40) (Bishop, 2003, 2016).

Social Responsiveness Scale (SRS-2)

The SRS-2 is a 65-item screening instrument for dimensions of interpersonal and stereotyped behaviours related to ASD, divided into five treatment scales. Raw scores are converted into scaled scores (M = 50, SD = 10), based on the country- and sex-normed tables for children between 4-18 years in Belgium and 4-16 years in the USA. Three summary scales are calculated: Social

Communication and Interaction (SCI), based on the first four treatment scales; Restricted Interests and Behaviour (RIB), which is the same as the fifth treatment scale, and the Total *T*-score, based on all five treatment scales. Higher *T*-scores refer to more severe social problems. *T*-scores of 61 – 75 (pc 1.2 – 16) are interpreted as mild-moderate impairments, whereas *T*-scores > 75 (pc 1.2) refer to severe impairments in social responsiveness.

5.2.3 Statistical analyses

We used a prospective cross-sectional study design with independent comparisons and dimensional measures. First, we compared patients from Leuven and Geisinger for both 16p11.2 CNV groups. Independent Student's *t*- or Mann Whitney *U*-tests were performed to establish the cohort site-related differences across age and nine summary scores: CBCL internalising, CBCL externalising, CBCL total behavioural problems, CCC-2 GCC, CCC-2 PC, SRS-2 SCI, SRS-2 RIB, SRS-2 total *T*-score, and FSIQ. To look for deviations from the scores of typically developing peers, we performed one-sample Student's or Wilcoxon signed rank *t*-tests for both CNV groups.

Then, we performed cross-CNV comparisons. Due to the anticipated broad intra-group heterogeneity in participants with 16p11.2 CNVs, conventional statistical testing was complemented with descriptive analyses using a three-tiered method. Both CNV groups were compared at three different levels to fully grasp the data: 1) statistical testing at the group level, 2) percentage differences at the subgroup level and 3) delineation of (un)expected individual trends (Olsson, 2005). At the group level, cross-CNV comparisons were conducted using independent *t*-tests for the same nine summary scores with Cohen's *d* or rank biserial correlation *r* as effect size. At the subgroup level, proportions of children with 16p11.2 CNVs showing borderline to clinically relevant problems were calculated, using the corresponding cut-off scores for each measure (CBCL borderline to clinical problems: internalising/externalising/total > 59, CCC-2 mild-moderate to severe problems: GCC > 104, PC > 53, SRS-2 mild-moderate to severe problems: SCI/RIB/total > 60, FSIQ < 70). Next, proportion differences were determined using the Fisher's exact test with Odds ratio as effect size.

Regarding the subscales, in agreement with other studies (Kim et al., 2020, Van Den Heuvel et al., 2017, Verbesselt et al., 2022), participants were considered as having social-communicative problems if their scores differed more than one *SD* from the norm group average (CCC-2 subscales > 13, SRS- 2

subscale > 60, pc < 16). For the subscale scores on the CBCL, cut-offs for borderline to clinical behavioural problems were based on the manual (CBCL subscales > 64, pc < 8). At the individual level, exploratory general linear models were carried out with age and FSIQ as covariates and inheritance pattern, sex, cohort site and SES as factors to determine the influence of these confounding variables on three main summary scores (CBCL total, CCC-2 GCC, SRS-2 total). Given the different genotype (duplication-deletion), statistical analyses were performed for each CNV separately.

Finally, we conducted Pearson correlation analyses to investigate the association between behavioural, social-communicative skills and cognitive functioning. We compared the summary scores with the least overlap in questions/domains, in particular the CBCL total *T*-score, CCC-2 GCC, SRS RIB and FSIQ. For correlation analyses, the correlation coefficient was used as effect size with correlations of 0.1-0.3, 0.3-0.5, >0.5 interpreted as small, moderate and large effects respectively (Paul, 2010). Bonferroni corrections were applied for all analyses to reduce type I-errors. Statistical testing was carried out using JASP version 0.16.4 (JASP Team, 2022) and R 4.2.1 (R core team, 2017, 2018).

5.3 Results

5.3.1 Cohort site differences

The distribution of sex and ASD diagnoses was not significantly different for 16p11.2 CNVs across cohort sites, although, qualitatively, there was an increased ASD prevalence in the two Leuven groups. Similarly, the eight summary scores did not significantly differ across cohort sites (Supplementary Figure 5.1/Supplementary Table 5.2). Remarkably, a significantly higher variability was found for the GCC ($F = 17.698$, $p < 0.001$) and PC ($F = 19.230$, $p < 0.001$) in the Geisinger cohort with a wider range of scores. In Geisinger, FSIQ scores were, on average, 8-10 points higher than in Leuven for deletions and duplications, respectively. Since statistical tests revealed no relevant differences, the results of both cohort sites were combined for the following analyses.

5.3.2 16p11.2 CNVs compared to the normative sample

Figure 1 depicts the normal distribution of FSIQ scores, displaying a downward shift of approximately 27 FSIQ points (≈ 1.8 *SD*) in both CNV groups compared to the normal distribution in the general population ($M = 100$, $SD = 15$). The normal distributions of participants with 16p11.2 CNVs are substantially overlapping. Compared to the normative sample, one sample *t*-tests revealed statistically significantly lower scores in both CNV groups for all summary scores ($p < 0.001$) with large effect sizes ($d > 0.843$, $r > 0.946$), except for CBCL externalising problems in 16p11.2DS ($p = 0.095$) (Supplementary Figure 5.2 /Supplementary Table 5.3).

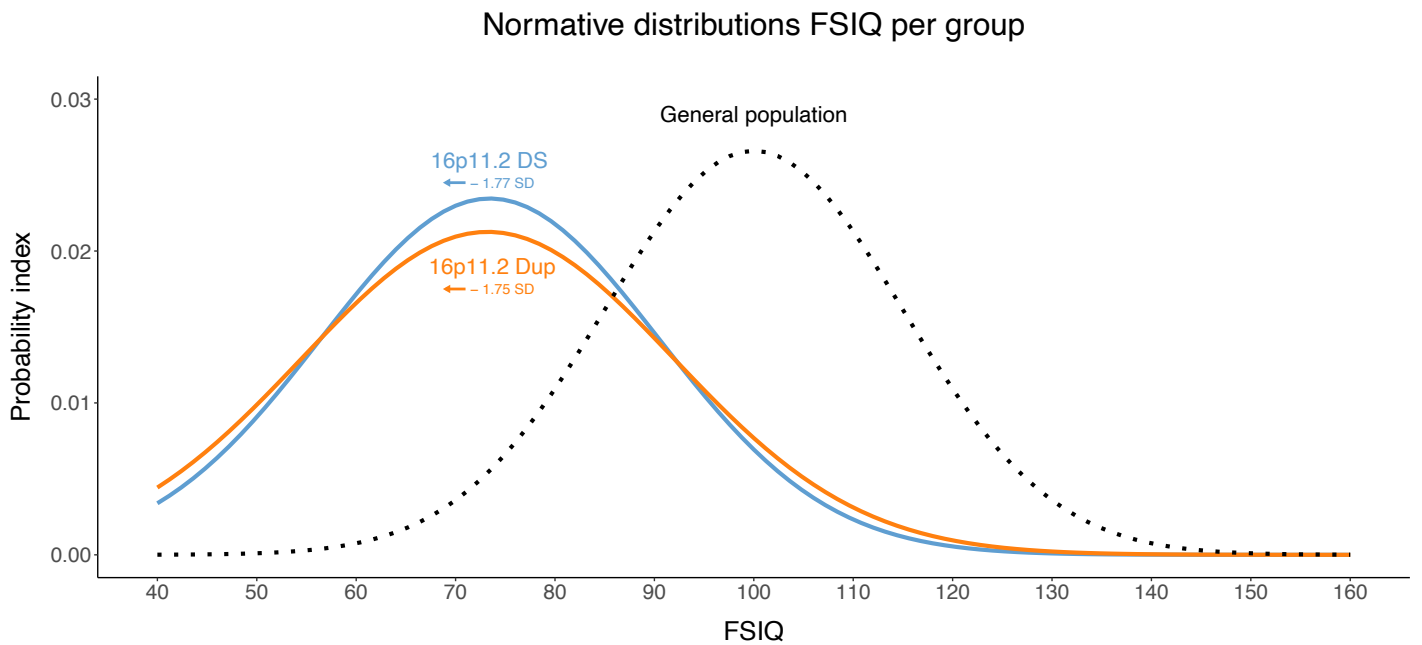


Figure 5.1 – Normative distributions FSIQ per group.
The dotted line represents the normal distribution of the norm group (M = 100, SD = 15). *SD* shifts are determined relative to the normative sample.

5.3.3 Quantitative and qualitative cross-CNV comparisons

Mean scores demonstrate behavioural scores within the normal range for children with 16p11.2DS, compared to clinically elevated behavioural scores in children with 16p11.2Dup (Table 5.2). Furthermore, children with 16p11.2DS experience, on average, mild-moderate social-communicative problems, opposed to severe social-communicative problems in 16p11.2Dup. At the group level, children with 16p11.2Dup scored statistically significantly higher on CBCL externalising, and total behavioural *T*- scores than children with 16p11.2DS with large effect sizes (Table 5.2). Comparisons of proportions at the subgroup level indicated that children with 16p11.2Dup showed statistically significantly more externalising behavioural problems than children with 16p11.2DS.

Boxplots for subscale scores across CBCL, CCC-2 and SRS-2 are displayed in Figure 5.2, showing a wide range of scores for most subscales across 16p11.2 CNVs. Most striking is the difference on the CBCL subscale aggressive behaviours between 16p11.2DS and 16p11.2Dup scores. For the CBCL syndrome scales, the most reported problems in children with 16p11.2DS were social problems (50%), attention problems and being withdrawn/depressed (43%), and thought problems (30%), whereas the most reported problems in children with 16p11.2Dup were at much higher rates: aggressive behaviours in 83%, attention problems in 78%, and social problems and thought problems in 71%. Descriptive statistics and proportions for all subscales are available in Supplementary Table 5.4.

Table 5.2 – Presence and severity of behavioural, social-communicative and cognitive problems in 16p11.2 CNVs

	16p11.2DS *N* *M (SD)* % with problems	**16p11.2Dup** *N* *M (SD)* % with problems	**Statistical outcomes group level** Independent samples *t*-test (*t*/*u* = , *p* = , *d*/*r* =)	**Statistical outcomes subgroup level** Fisher's exact (*p* = , *OR* =)
CBCL internalising *T*-score				
N	42	18		
M (SD)	59.38 (11.13)	66.06 (8.52)	*t* = -2.271	
Range	33.00 – 80.00	52.00 – 84.00	*p* = 0.027	*p* = 0.046
% with problems	47.62%	77.78%	*d* = -0.640	*OR* = 1.326
CBCL externalising *T*-score				
N	42	18		
M (SD)	53.00 (11.63)	67.94 (10.98)	*t* = -4.715	
Range	33.00 – 76.00	41.00 – 88.00	*p* < 0.001*	*p* < 0.001*
% with problems	28.57%	88.89%	*d* = -1.328	*OR* = 2.938
CBCL total *T*-score				
N	42	18		
M (SD)	59.74 (10.46)	70.17 (8.73)	*t* = -3.707	
Range	38.00 – 82.00	51.00 – 82.00	*p* < 0.001*	*p* = 0.009
% with problems	52.38%	88.89%	*d* = -1.044	*OR* = 1.955
CCC-2 GCC SS				
N	39	15		

M (SD)	111.49 (18.63)	118.00 (16.47)	*U* = 259.000	
Range	64.00 – 139.00	86.00 – 150.00	*p* = 0.523	*p* = 1.000
% with problems	69.23%	73.33%	*r* = -0.115	*OR* = 0.197
CCC-2 PC SS				
N	39	15		
M (SD)	54.90 (9.61)	59.93 (7.25)	*U* = 228.500	
Range	34.00 – 69.00	49.00 – 75.00	*p* = 0.219	*p* = 0.508
% with problems	66.67%	80.00%	*r* = -0.219	*OR* = 0.681
SRS-2 SCI *T*-score				
N	45	21		
M (SD)	73.04 (17.99)	79.86 (13.54)	*t* = -1.541	
Range	40.00 – 111.00	54.00 – 100.00	*p* = 0.128	*p* = 0.117
% with problems	71.11%	90.48%	*d* = -0.407	*OR* = 1.333
SRS-2 RIB *T*-score				
N	45	21		
M (SD)	69.73 (19.01)	84.00 (19.98)	*t* = -2.794	
Range	41.00 – 113.00	51.00 -134.00	*p* = 0.007	*p* = 0.037
% with problems	64.44%	90.48%	*d* = -0.738	*OR* = 1.635
SRS-2 total *T*-score				
N	45	21		
M (SD)	73.38 (18.43)	81.71 (15.07)	*t* = -1.808	
Range	41.00 – 115.00	56.00 – 107.00	*p* = 0.075	*p* = 0.070
% with problems	68.89%	90.48%	*d* = -0.478	*OR* = 1.438

FSIQ				
N	36	18		
M (SD)	73.44 (17.01)	73.71 (18.90)	t = -0.050	
Range	45.00 – 106.00	48.00 – 101.00	p = 0.960	p = 0.384
% with ID	38.89%	52.94%	d = 0.033	*OR* = 0.559

Note. Statistical outcomes: p-value; * significant after Bonferroni correction at $p < 0.006$; t-value or U-value. CBCL (norm group average = 50, cut-off: >59 = borderline, ≥64 = clinical); GCC, general communication composite (norm group average = 80, cut-off: >104 = mild-moderate, ≥117 = severe); PC, pragmatic composite (norm group average = 40, cut-off: >53 = mild-moderate, ≥60 = severe); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour (norm group average = 50, cut-off: >60 = mild–moderate, ≥76 severe); FSIQ, full-scale intelligence quotient (norm group average = 100, cut-off: <70 = mild ID, <55 = moderate ID).

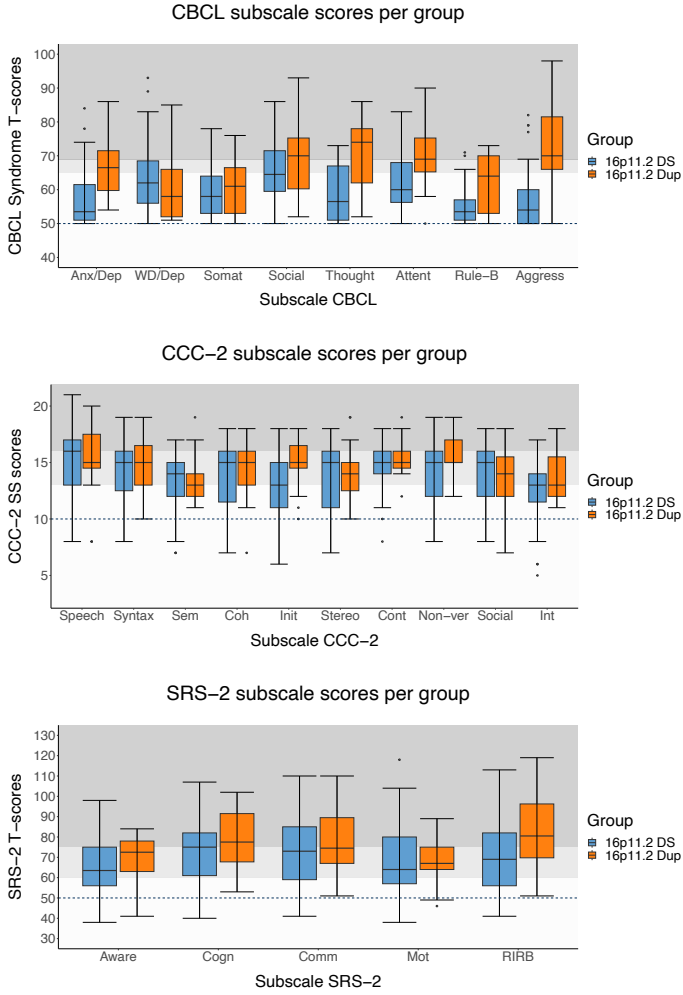


Figure 5.2 – Boxplots for CBCL (*M* = 50, *SD* = 10), CCC-2 (*M* = 10, *SD* = 3) and SRS-2 (*M* = 50, *SD* = 10) across subscales.
The dashed lines show norm group averages. The grey zones indicate the severity of the problems based on clinical cut-off scores for CBCL, CCC-2 and SRS-2; the darker the grey, the more severe the difficulties: mild–moderate/borderline = light grey zone and severe/clinical = darker grey zone. Abbreviations: CBCL subscales: Anx/Dep, Anxious/Depressed; WD/Dep, Withdrawn/Depressed; Somat, Somatic Complaints; Social, Social Problems; Thought, Thought Problems; Attent, Attention Problems; Rule-B, Rule-Breaking Behaviours; Aggress, Aggressive Behaviours. CCC-2 subscales: Speech; Syntax; Sem, Semantics; Coh, Coherence; Init, Inappropriate Initiation; Stereo, Stereotyped Language; Cont, Use of Context; Non-ver, Non-verbal Communication; Social, Social relations; Int, Interests. SRS-2 subscales: Aware, Social Awareness; Cogn, Social Cognition; Comm, Social Communication; Mot, Social Motivation; RIRB, Restricted Interests and Repetitive Behaviours.

Regarding CCC-2 subscale scores, parents of children with 16p11.2DS mostly reported problems with use of context (77%), coherence (69%) and speech production (67%), whereas parents of children with 16p11.2Dup mostly reported problems with use of context (93%), speech production, inappropriate initiation and non-verbal communication (80%), and syntax (73%). For SRS-2 subscales, parents of children with 16p11.2DS had most concerns about social cognition (80%), social communication (69%) and restricted interests and repetitive behaviours (64%), whereas parents of children with 16p11.2Dup worried most about social cognition, social communication, social motivation, restricted interests and repetitive behaviours (90%), and social awareness (85%).

5.3.4 Impact of confounding factors

The results for the exploratory general linear models are displayed in Supplementary Table 5.5. Within both 16p11.2 CNV groups, none of the models or factors reached significance.

5.3.5 Association between behavioural, social-communicative skills and IQ in 16p11.2 CNVs

Within the 16p11.2DS group, strong and significant correlations were found between behavioural (CBCL total), social responsiveness (SRS RIB) and communicative (CCC-2 GCC) skills ($r > 0.6$, $p < 0.001$). Cognitive functioning (FSIQ) was only significantly correlated with the GCC ($r = -0.508$, $p = 0.03$; Supplementary Table 5.6). Within the 16p11.2Dup group, we observed large and significant correlations ($r > 0.6$, $p < 0.006$) between the skills measured by the three questionnaires. No significant associations were found between these skills and FSIQ ($r < 0.6$, $p > 0.027$). Correlation plots in Supplementary Figure 5.3 show the associations between these four variables. Figure 5.3 shows the comorbidity between behavioural, social responsiveness and communicative problems based on the (sub)clinical cut-offs for the three questionnaires. Borderline or clinically elevated scores on all three questionnaires were obtained by 44% (16/36) of children with 16p11.2DS and 64% (9/14) of children with 16p11.2Dup.

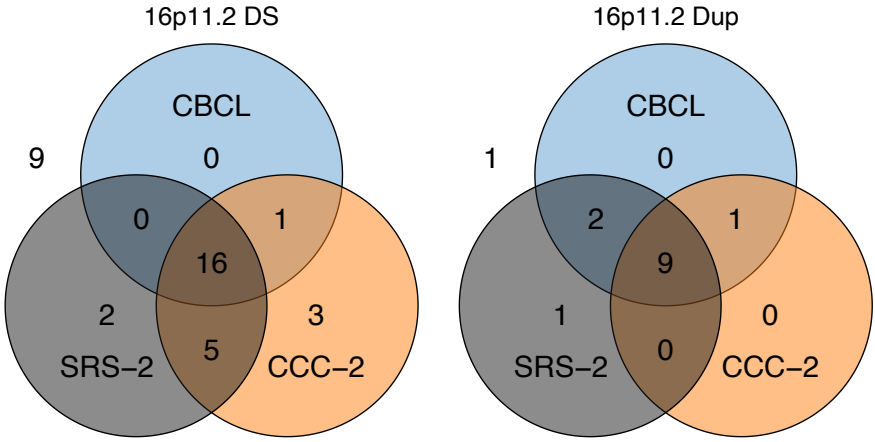


Figure 5.3 – Comorbidity in 16p11.2DS – (sub)clinical scores on CBCL total, CCC-2 GCC and SRS-2 RIB.
Participants with (sub)clinically elevated scores across CBCL total *T*-scores (cut-off: >59 = borderline, ≥64 = clinical), CCC-2 GCC, general communication composite (cut-off: >104 = mild-moderate, ≥117 = severe) and SRS-2 RIB, Restricted Interests and Repetitive Behaviour *T*-scores (cut-off: >60 = mild–moderate, ≥76 severe). The number outside the Venn diagram refers to the remaining participants for whom all three questionnaires were completed by the parents.

5.4 Discussion

The aim of the current study was to investigate the prevalence, nature and severity of, and associations between behavioural and social-communicative skills of school-aged children with 16p11.2 CNVs, using a standardised protocol with both direct and indirect measures and combining data from two different cohort sites. In general, both cohort sites were comparable, as was expected based on similar referral patterns for genetic testing. Qualitatively, similar FSIQ differences with lower average FSIQ scores in European cohorts compared to USA cohorts were reported before (D'Angelo et al., 2016). Variations between European and USA cohorts may arise from differences in instruments used and access to genetic testing, which can vary depending on the healthcare system in place. The higher variability in communicative scores in 16p11.2DS from the USA might be caused by more diverse communication profiles in the USA cohort or by parents with more extreme opinions on their children's communication skills. Cultural or spoken language differences and different parental expectations on communication might also contribute to discrepancies between cohorts.

Comparisons to the norm group point to the high prevalence of behavioural, social-communicative and cognitive impairments in both 16p11.2 CNV groups compared to the normative sample, which is in line with previous studies (Green Snyder et al., 2016; Hanson et al., 2015; Kim et al., 2020; Moreno-De- Luca et al., 2015; Niarchou et al., 2019; Taylor et al., 2023). The average FSIQ of 73 in both 16p11.2 CNVs was at the lower end of what has been described in literature before, with FSIQ predominantly within the borderline range (Chawner et al., 2021; Green Snyder et al., 2016; Hanson et al., 2015; Jutla et al., 2020; Modenato et al., 2021; Moreno-De-Luca et al., 2015; Taylor et al., 2023). The relatively lower FSIQ scores might be related to the fact that the current sample is a clinically ascertained cohort, potentially covering only the more severe end of the phenotypic spectrum of 16p11.2 CNVs. The use of different instruments, various age ranges and limited sample sizes might also contribute to these differences.

Behavioural problems were elevated in 52% of patients with 16p11.2DS, compared to 89% of children with 16p11.2Dup. Consistent with the findings of Green Snyder et al. (2016), children with 16p11.2Dup exhibited clinical behavioural problems, whereas those with 16p11.2DS had average CBCL summary scores falling within the normal range. In children with 16p11.2 CNVs, all mean CCC-2 and SRS-2 summary scores met clinical cut-offs for mild-moderate to severe social-communicative issues, confirming previous research (Green Snyder et al., 2016; Hanson et al., 2015; Moreno-De-Luca et al., 2015; Smith et al., 2022; Taylor et al., 2023) and indicating that although not all individuals with 16p11.2 CNVs meet diagnostic criteria for ASD, almost all demonstrate certain autistic features (Green Snyder et al., 2016; Taylor et al., 2021). Parents reported communicative and social responsiveness problems in most patients with 16p11.2DS (69% for both) and 16p11.2Dup (73% and 90% respectively), which is in line with the proportions found by Kim et al. (2020). The fact that almost all children had delayed emergence of speech-language milestones and most of them needed speech-language therapy might indicate that the proportion of communicative problems reported by parents is an underestimation of the true prevalence of communicative impairments, which was suggested before in other CNVs (Verbesselt, Van Den Heuvel, et al., 2022). However, due to missing data on certain developmental variables in patients from Geisinger, the elevated rates of delays and children receiving speech-language therapy might not be representative of the entire cohort.

Cross-CNV comparisons revealed significantly more externalising behaviours in children with 16p11.2Dup. These findings might be partially explained by the relatively low level of reported externalising behaviours in children with 16p11.2DS, which has been reported before by Hanson et al. (2015), whereas children with 16p11.2Dup showed equally elevated internalising and externalising behaviours. Qualitatively, children with 16p11.2Dup were reported to have higher mean SRS-2 scores, indicating more severe social responsiveness problems, which was in line with previous studies (Green Snyder et al., 2016; Moreno-De-Luca et al., 2015; Smith et al., 2022; Taylor et al., 2023) and could be related to the relatively high proportion of ASD diagnoses in children with 16p11.Dup, which was also the case in the current cohort (11/20,55%). The overlapping features in both 16p11.2 CNVs with sometimes even more severe deficits in the duplication group seem to be unique to the 16p11.2 locus, since for other CNVs, such as 22q11.2 deletion syndrome/duplication or 7q11.23 deletion/duplication, duplications were mostly associated with milder phenotypes compared to deletions within the same chromosomal region (Goldenberg, 2018; Verbesselt et al., 2023; Verbesselt, Zink, et al., 2022).

Interestingly, at the subscale level, the most reported problems for both CNV groups were across similar behavioural domains, including attention, thought and social problems, but consistently at lower rates for the deletion group. Only withdrawn/depressed behaviours were more prevalent in 16p11.2DS. The high rates of aggressive behaviours in 16p11.2Dup were remarkable, since only lower rates were reported before (Bernier et al., 2017; Green Snyder et al., 2016; Rosenfeld et al., 2010). Regarding the CCC-2 subscales, speech production problems, and especially speech motor difficulties such as childhood apraxia of speech, have been previously identified as a core feature of 16p11.2DS occurring in approximately 80% (Fedorenko et al., 2016; Mei et al., 2018). However, in 16p11.2Dup, speech production problems have so far only been mentioned explicitly by Steinman et al. (2016). Characterisation through direct speech instruments is needed to further elucidate the speech production spectrum in both CNVs. Remarkably, the most reported communicative concerns across both CNV groups were problems with the use of context in almost all children with 16p11.2Dup (93%) and the majority of children with 16p11.2DS (77%). Therefore, this pragmatic language domain might require specific focus in language therapy. Direct language tests assessing structural, semantic and pragmatic language skills should further clarify the specific deficits across these language domains. Especially in the 16p11.2Dup, all social

responsiveness traits seemed equally affected, and no specific pattern of autistic features stood out. None of the confounding factors had a significant influence on the results, indicating that behavioural and social-communicative impairments were reported independent of age, IQ, SES, sex, cohort site or inheritance pattern. However, future research with larger samples is required to further explore the influence of these and other potentially confounding factors, such as medical comorbidities (e.g. epilepsy, BMI).

Strong and significant associations were found between autistic mannerisms, behavioural and communicative skills, mostly independent of cognitive functioning. These results point to the high comorbidity between behavioural and social-communicative skills. This finding is consistent with previous research on children with 22q11.2 deletion syndrome (22q11.2DS), where positive associations were reported between language, social responsiveness (SRS-2) and behavioural (CBCL) scores, while overall cognitive abilities showed weaker associations (Chawner et al., 2023). The results suggest that cognitive functioning might contribute to, but cannot entirely account for the occurrence of autistic mannerisms, communicative, and behavioural problems in these CNV groups. This aligns with previous research using a categorical approach, which indicated that ID and other NDDs represent distinct outcomes of 22q11.2DS (Green et al., 2009; Niarchou et al., 2014).

Given the fact that only summary scores with minimal to no overlap in questions/domains were compared, these robust associations may reflect comorbid difficulties across social responsiveness, communicative and behavioural domains, suggesting the co-development of distinct underlying constructs in 16p11.2 CNVs. However, the study from Chawner et al. (2023) reported positive associations between almost all subscales of the SRS, CBCL, language, motor and various neurocognitive skills, implying that these characteristics could potentially signal an underlying transdiagnostic construct. Further investigations involving larger cohorts are warranted to elucidate the underlying factors contributing to behavioural and cognitive traits in early childhood among individuals with these CNVs. Children with both social-communicative and behavioural problems are even more vulnerable and need closer follow-up and care in the future. Healthcare professionals should be aware of the fact that children might still experience behavioural and social-communicative problems in the absence of cognitive impairments. On the other hand, children with ID do not necessarily develop behavioural and social-communicative issues. To conclude, the current cohort of children with 16p11.2

CNVs showing heterogeneous profiles seems to be a representative sample of clinically referred patients and adds to the current knowledge on the neurodevelopmental phenotype of these CNVs.

5.4.1 Strengths, limitations and future

Key strengths of the current study are the prospective nature, and the cross-CNV comparisons in school-aged children with 16p11.2 CNVs. Complementing a dimensional approach with categorical data combines the benefits of both and gives health care professionals insights into the presence, type and severity of hallmark neurodevelopmental features in school-aged children with 16p11.2 CNVs, that should be addressed in therapy (Moreno-De-Luca et al., 2015). The collaboration between Leuven and Geisinger allowed us to achieve relatively large sample sizes and achieve more statistical power.

Children with additional pathogenic CNVs were excluded from the sample. However, the inclusion of children with variants of unknown significance variants might still have had some influence on the phenotype. Since not all children were tested through exome or whole genome sequencing, we could not rule out with certainty the presence of additional pathogenic variants that might have affected the phenotype, especially in children with more severe phenotypes. By including only index patients who were referred for medical or neurodevelopmental concerns, the current cohort most likely represented the more severe end of the phenotype. Cascade testing for carrier relatives might contribute to the delineation of the phenotype of non-proband carriers and provide insight in the complete phenotypic spectrum in 16p11.2 CNVs.

Standardised parental questionnaires are a valuable initial measure to expand the knowledge on neurodevelopmental characteristics in 16p11.2 CNVs (Bennetts et al., 2016; Bishop & McDonald, 2009; Garibaldi et al., 2021; Van Roy et al., 2010). However, indirect methods might also introduce bias, since parents might have different perspectives on their children compared to clinically trained researchers and adoptive or foster parents might not be able to answer all questions related to the neonatal period and early development. In addition, more complex family settings with bilingualism and other context-related factors (e.g. trauma) might have played a role in the social-communicative and behavioural outcomes. The environmental factor SES was not found to have a significant influence, but data in many children were missing. In addition to cross-sectional characterisation with indirect instruments such as questionnaires, longitudinal studies with in-person assessments are required to establish the evolution of

behavioural and social-communicative features in 16p11.2 CNVs across the lifespan.

5.5 Conclusion

This multi-site study investigates the association between behavioural, social-communicative skills and cognitive functioning in children aged 6-17 years with 16p11.2 CNVs. School-aged children with 16p11.2 CNVs show high rates of behavioural, social-responsiveness and communicative problems compared to the typical population. These findings point to the high prevalence of autistic traits and diagnoses in these CNV populations. Moreover, there is a high comorbidity between behavioural and social- communicative problems. Patients with difficulties in both domains are vulnerable and need closer clinical follow-up and care.

5.6 Supplementary material

Supplementary Table 5.1 – Administered IQ tests across cohort sites

	IQ-test	Reference
Leuven	Dutch version of the Wechsler Intelligence Scale for Children – Fifth Edition (WISC-V-NL)	Wechsler, D. (2014). Wechsler intelligence scale for children (5th ed.): WISC-V. Pearson. Hendriks, M.P.H, van der Heijden, P.T., van Dijk, M., Ruiter, S., & van der Vlugt, H. (2019). De Wechsler intelligentietest voor kinderen 5e editie: WISC-V. Neuropraxis, 23(3), 63–71. https://doi.org/10.1007/s12474-019-00224-4
Geisinger	Kaufmant Brief Intelligence Test – Second Edition (KBIT-2)	Kaufman, A.S., & Kaufman, N.L. (2004). Kaufman Brief Intelligence Test – Second Edition (KBIT-2). American Guidance Service
	Wechsler Preschool and Primary Scale of Intelligence – Fourth Edition (WPPSI-IV)	Wechsler, D. (2012). Wechsler Preschool and Primary Scale of Intelligence – Fourth Edition (WPPSI-IV). Pearson
	Differential Ability Scales – Second Edition (DAS-II)	Beran, T.N., & Elliott, C.D. (2007). Differential Ability Scales – Second Edition (DAS-II). Canadian Journal of School Psychology, 22(1), 128-132. https://doi.org/10.1177/0829573507302967 Elliott, C. D. (2007). Differential Ability Scales (2nd ed.). San Antonio, TX: Harcourt Assessment
	Wechsler Abbreviated Scale of Intelligence – Second Edition (WASI-II)	Wechsler, D. (2011). Wechsler Abbreviated Scale of Intelligence – Second Edition (WASI-II). NCS Peason

	Kaufman Asessment Battery for Children – Second Edition (KABC-II)	Kaufman, A.S., & Kaufman, N.L. (2004). Kaufman Asessment Battery for Children – Second Edition (KABC-II). American Guidance Service.
	Primary Test of Nonverbal Intelligence (PTONI)	Ehrler & McGhee (2008). Primary Test of Nonverbal Intelligence (PTONI). PRO-E.

Supplementary Table 5.2 – Cohort site differences

	16p11.2DS			**16p11.2Dup**		
	Leuven	**Geisinger**	**Statistical outcomes**	**Leuven**	**Geisinger**	**Statistical outcomes**
	N *M (SD)* Median Min - Max	*N* *M (SD)* Median Min - Max	Independent samples *t*-test (*t*/*u* = , *p* = , *d*/*r* =)	*N* *M (SD)* Median Min - Max	*N* *M (SD)* Median Min - Max	Independent samples *t*-test (*t*/*u* = , *p* = , *d*/*r* =)
Age (in months): *N*	24	23		11	10	
M (SD)	10.11 (3.2)	12.0 (3.2)	*t* = -1.161	10.5 (2.11)	12.3 (3.3)	*t* = -1.350
Median	10.2	12.5	*p* = 0.252	9.7	13.5	*p* = 0.193
Min - Max	6.7 – 16.11	6.2 – 17.1	*d* = -0.339	6.1 – 14.7	6.1 – 16.7	*d* = -0.590
CBCL internalising *T*-score: *N*	24	18		11	7	
M (SD)	59.17 (12.01)	59.67 (10.18)	*t* = -0.142	62.82 (7.94)	71.14 (7.18)	*t* = -2.248
Median	58.50	58.50	*p* = 0.888	65.00	70.00	*p* = 0.039
Min - Max	33.00 – 80.00	41.00 – 76.00	*d* = -0.044	52.00 – 72.00	61.00 – 84.00	*d* = -0.571

CBCL externalising *T*-score: *N*	24	18		11	7	
M (SD)	52.25 (11.03)	54.00 (12.04)	t = -0.489	64.64 (11.33)	73014 (8.73)	t = -1.687
Median	55.00	51.500	p = 0.627	66.00	70.00	p = 0.111
Min - Max	33.00 – 76.00	37.00 – 76.00	d = -0.153	41.00 – 80.00	62.00 – 88.00	d = 0.111
CBCL total *T*-score: *N*	24	18		11	7	
M (SD)	59.63 (10.44)	59.89 (10.79)	U = 203.000	67.36 (9.15)	74.57 (6.29)	t = -1.819
Median	59.00	63.00	p = 0.750	71.00	73.00	p = 0.088
Min - Max	38.00 – 82.00	38.00 – 71.00	r = -0.060	51.00 – 81.00	65.00 _ 82.00	d = -0.880
CCC-2 GCC SS: *N*	24	15		11	4	
M (SD)	116.33 (11.10)	103.73 (25.19)	U = 224.500	117.82 (15.24)	118.50 (22.16)	t = -0.068
Median	120.00	105.00	p = 0.203	119.00	110.50	p = 0.947
Min - Max	90.00 – 129.00	64.00 – 139.00	r = 0.247	86.00 – 140.00	103.00 – 150.00	d = -0.040
CCC-2 PC SS: *N*	24	15		11	4	
M (SD)	57.29 (5.75)	51.07 (13.06)	U = 218.500	59.73 (6.20)	60.50 (10.79)	t = -0.176
Median	59.50	54.00	p = 0.272	61.00	59.00	p = 0.863
Min - Max	46.00 – 65.00	34.00 – 69.00	r = 0.214	49.00 – 71.00	49.00 – 75.00	d = -0.103
SRS-2 SCI *T*-score: *N*	24	21		11	10	
M (SD)	78.42 (16.80)	66.91 (17.71)	t = 2.237	78.73 (16.88)	81.10 (9.34)	t = -0.393
Median	77.00	71.00	p = 0.031	77.00	79.00	p = 0.699
Min – Max	56.00 – 111.00	40.00 – 102.00	d = 0.668	54.00 – 100.00	71.00 – 96.00	d = -0.172
SRS-2 RIB *T*-score: *N*	24	21		11	10	
M (SD)	73.38 (19.06)	65.57 (18.52)	t = 1.388	85.18 (26.61)	82.70 (12.45)	t = 0.278
Median	70.00	68.00	p = 0.172	79.00	82.50	p = 0.784

Min - Max	42.00 – 113.00	41.00 – 100.00	d = 0.415	51.00 – 134.00	64.00 – 100.00	d = 0.121
SRS-2 total *T*-score: *N*	24	21		11	10	
M (SD)	78.88 (17.49)	67.10 (17.82)	t = 2.234	81.09 (19.04)	82.40 (10.04)	U = 51.000
Median	77.00	73.00	p = 0.031	78.00	81.00	p = 0.805
Min - Max	56.00 – 115.00	41.00 – 101.00	d = 0.668	56.00 – 107.00	70.00 – 98.00	r = -0.073
Full-scale IQ: *N*	23	13		11	6	
M (SD)	70.91 (14.89)	77.85 (19.97)	t = -1.185	69.73 (20.15)	79.67 (15.44)	t = -1.047
Median	73.00	77.00	p = 0.244	66.00	82.50	p = 0.312
Min - Max	45.00 – 99.00	45.00 – 106.00	d = -0.411	48.00 – 100.00	61.00 – 101.00	d = -0.531

Note. Statistical outcomes: *p*-value; *significant after Bonferroni correction $p < 0.005$; *t*-value or *U*-value. Abbreviations. CBCL (norm group average = 50, cut-off: >59 = borderline, ≥64 = clinical); GCC, general communication composite (norm group average = 80, cut-off: >104 = mild-moderate, ≥117 = severe); PC, pragmatic composite (norm group average = 40, cut-off: >53 = mild-moderate, ≥60 = severe); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour (norm group average = 50, cut-off: >60 = mild–moderate, ≥76 severe

Supplementary Table 5.3 – CNV groups compared to the normative sample

	16p11.2DS *N* *M (SD)* Median	**Statistical outcomes group level** One sample *t*-test *(t/V = , p = , d/r =)*	**16p11.2Dup** *N* *M (SD)* Median	**Statistical outcomes group level** One sample *t*-test *(t/V = , p = , d/r =)*
CBCL internalising *T*-score:				
N	42	t = 5.463	18	t = 7.992
M (SD)	59.38 (11.13)	$p < 0.001$*	66.06 (8.52)	$p < 0.001$*

Median	58.50	$d = 0.843$	68.50	$d = 1.884$
CBCL externalising *T*-score:				
N	42	$t = 1.711$	18	$t = 6.935$
M (SD)	53.00 (11.63)	$p = 0.095$	67.94 (10.98)	$p < 0.001$*
Median	54.50	$d = 0.264$	68.00	$d = 1.635$
CBCL total *T*-score:				
N	42	$t = 6.033$	18	$t = 9.797$
M (SD)	59.74 (10.46)	$p < 0.001$*	70.17 (8.73)	$p < 0.001$*
Median	60.50	$d = 0.931$	71.00	$d = 2.309$
CCC-2 GCC SS:				
N	39	$V = 729.500$	15	$t = 8.938$
M (SD)	111.49 (18.63)	$p < 0.001$*	118.00 (16.47)	$p < 0.001$*
Median	120.00	$r = 0.969$	118.00	$d = 2.308$
CCC-2 PC SS:				
N	39	$V = 759.000$	15	$t = 10.655$
M (SD)	54.90 (9.61)	$p < 0.001$*	59.93 (7.25)	$p < 0.001$*
Median	58.00	$r = 0.946$	61.00	$d = 2.751$
SRS-2 SCI *T*-score:				
N	45	$t = 8.592$	21	$t = 10.108$
M (SD)	73.04 (17.99)	$p < 0.001$*	79.86 (13.54)	$p < 0.001$*
Median	75.00	$d = 1.281$	77.00	$d = 2.206$

SRS-2 RIB *T*-score:				
N	45	$t = 6.963$	21	$t = 7.797$
M (SD)	69.73 (19.01)	$p < 0.001$*	84.00 (19.98)	$p < 0.001$*
Median	69.00	$d = 1.038$	82.00	$d = 1.701$
SRS-2 total *T*-score:				
N	45	$t = 8.510$	21	$t = 9.644$
M (SD)	73.38 (18.43)	$p < 0.001$*	81.71 (15.07)	$p < 0.001$*
Median	75.00	$d = 1.269$	79.00	$d = 2.104$
Full-scale IQ:				
N	36	$t = -9.408$	18	$t = -5.881$
M (SD)	73.44 (17.01)	$p < 0.001$*	73.71 (18.90)	$p < 0.001$*
Median	75.00	$d = -1.568$	66.00	$d = -1.426$

Note. Statistical outcomes: *p*-value; *significant after Bonferroni correction $p < 0.0055$; *t*-value or *U*-value. Abbreviations. CBCL (norm group average = 50, cut-off: >59 = borderline, ≥64 = clinical); GCC, general communication composite (norm group average = 80, cut-off: >104 = mild-moderate, ≥117 = severe); PC, pragmatic composite (norm group average = 40, cut-off: >53 = mild-moderate, ≥60 = severe); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour (norm group average = 50, cut-off: >60 = mild–moderate, ≥76 severe).

Supplementary Table 5.4 – Descriptive statistics subscale scores in both CNVs

		16p11.2DS			**16p11.2Dup**		
		N	*M (SD)*	% with problems	*N*	*M (SD)*	% with problems
CBCL	Internalising – Anxious/Depressed	42	57.00 (8.40)	14.29%	18	67.17 (9.82)	61.11%

	Internalising – Withdrawn/Depressed	40	63.85 (10.54)	42.50%	15	60.20 (9.99)	33.33%
	Internalising – Somatic Complaints	42	59.31 (8.31)	23.81%	18	60.44 (8.38)	33.33%
	Social Problems	38	65.18 (9.14)	50.00%	14	69.14 (11.69)	71.43%
	Thought Problems	38	59.05 (8.18)	31.58%	17	70.88 (10.14)	70.59%
	Attention Problems	42	62.17 (8.72)	42.86%	18	70.00 (10.22)	77.78%
	Externalising – Rule-Breaking Behaviour	38	55.24 (5.74)	10.53%	17	61.94 (8.57)	41.18%
	Externalising – Aggressive Behaviour	42	56.95 (9.06)	19.05%	18	72.00 (13.32)	83.33%
CCC-2	Speech	39	15.03 (3.59)	66.67%	15	15.20 (3.61)	80.00%
	Syntax	39	14.36 (2.93)	64.10%	15	14.93 (2.84)	73.33%
	Semantics	39	13.15 (2.60)	53.85%	15	13.60 (2.23)	40.00%
	Coherence	39	15.05 (2.72)	69.23%	15	14.33 (2.87)	60.00%
	Inappropriate Initiation	39	12.62 (2.80)	38.46%	15	14.87 (2.30)	80.00%
	Stereotyped Language	39	13.67 (3.02)	58.97%	15	14.33 (2.61)	66.67%
	Use of Context	39	15.56 (2.29)	76.92%	15	15.33 (1.68)	93.33%
	Non-verbal Communication	39	14.05 (2.91)	61.54%	15	15.40 (1.96)	80.00%
	Social Relations	39	13.97 (2.86)	61.54%	15	13.67 (3.06)	60.00%
	Interests	39	12.31 (3.03)	35.90%	15	13.67 (2.23)	46.67%
SRS-2	Social Awareness	45	62.60 (15.92)	53.33%	20	70.25 (10.92)	85.00%
	Social Cognition	45	72.29 (16.27)	80.00%	20	79.50 (14.58)	90.00%
	Social Communication	45	72.51 (18.12)	68.89%	20	76.50 (15.64)	90.00%
	Social Motivation	45	68.98 (18.95)	53.33%	20	69.15 (11.42)	90.00%
	Repetitive Behaviours/Restricted Interests	45	69.73 (19.01)	64.44%	21	84.00 (19.98)	90.48%

Supplementary Table 5.5 – Exploratory general linear models across 16p11.2 CNVs

16p11.2DS	**CCC-2 GCC**	**SRS-2 total**	**CBCL total**
	Estimate	Estimate	Estimate
	Standard error	Standard error	Standard error
	t =, p =	t =, p =	t =, p =
Intercept	E = 106.242	E = 42.301	E = 16.437
	SE = 18.269	SE = 33.834	SE = 16.963
	t = 5.815	t = 1.250	t = 0.969
	p < 0.001	p = 0.235	p = 0.353
Inheritance	E = -2.592	E = -0.836	E = 6.194
pattern_Inherited	SE = 70459	SE = 15.229	SE = 7.614
	t = -0.347	t = -0.055	t = 0.814
	p = 0.734	p = 0.957	p = 0.433
Cohort site_Geisinger	E = 3.686	E = -10.087	E = 2.199
	SE = 5.798	SE = 11.8462	SE = 7.726
	t = 0.636	t = -0.880	t = 0.285
	p = 0.536	p = 0.396	p = 0.781
Sex_Male	E = -1.140	E = -7.142	E = -11.647
	SE = 5.426	SE = 9.355	SE = 5.087
	t = -0.210	t = -0.763	t = -2.289
	p = 0.837	p = 0.460	p = 0.043
SES_middle	E = 15.186	E = 21.268	E = 11.587
	SE = 11.103	SE = 19.881	SE = 10.044
	t = 1.368	t = 1.070	t = 1.154
	p = 0.195	p = 0.306	p = 0.273
SES_high	E = 21.339	E = 27.264	E = 21.065
	SE = 10.999	SE = 19.881	SE = 10.479
	t = 1.940	t = 1.070	t = 2.010
	p = 0.074	p = 0.306	p = 0.070
Age	E = 0.062	E = 0.188	E = 0.160
	SE = 0.062	SE = 0.106	SE = 0.055
	t = 1.009	t = 1.773	t = 2.940
	p = 0.332	p = 0.102	p = 0.013

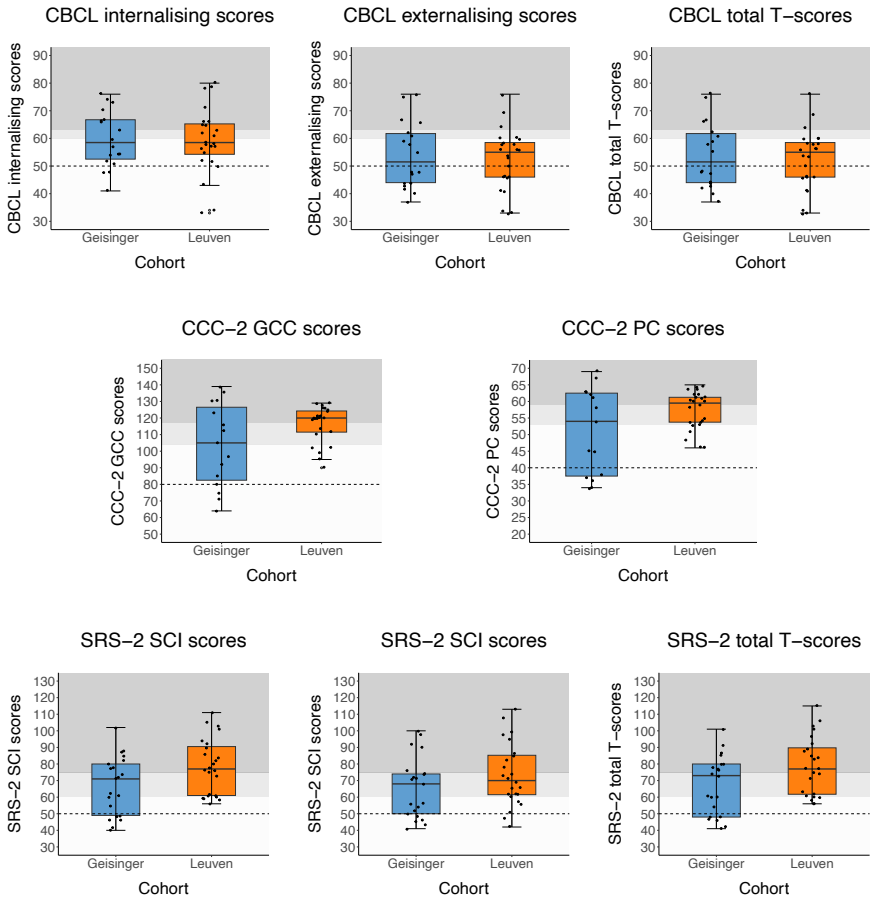
16p11.2Dup	**SRS-2 total**	**CBCL total**
	Estimate	Estimate
	Standard error	Standard error
	t =, *p* =	*t* =, *p* =
Intercept	*E* = 118.859	*E* = 84.017
	SE = 48.212	*SE* = 17.754
	t = 2.465	*t* = 4.732
	p = 0.245	*p* = 0.133
Inheritance pattern_Inherited	*E* = 20.622	*E* = 13.803
	SE = 31.588	*SE* = 8.790
	t = 0.653	*t* = 1.570
	p = 0.632	*p* = 0.361
Cohort site_Geisinger	*E* = -0.317	*E* = 7.744
	SE = 26.645	*SE* = 8.713
	t = -0.012	*t* = 0.889
	p = 0.992	*p* = 0.537
Sex_Male	*E* = -6.614	*E* = 2.551
	SE = 21.555	*SE* = 12.284
	t = -0.307	*t* = 0.208
	p = 0.810	*p* = 0.870
SES_middle	*E* = -4.710	*E* = 3.430
	SE = 32.290	*SE* = 7.891
	t = -0.146	*t* = 0.435
	p = 0.908	*p* = 0.739
SES_high	*E* = -6.377	/
	SE = 28.008	
	t = -0.228	
	p = 0.857	
Age	*E* = -0.070	*E* = -0.039
	SE = 0.188	*SE* = 0.096
	t = -0.371	*t* = -0.411
	p = 0.774	*p* = 0.752

Note. Too few observations for general linear model on CCC-2 GCC scores in 16p11.2Dup group

Supplementary Table 5.6 – Correlation analyses in 16p11.2 CNVs

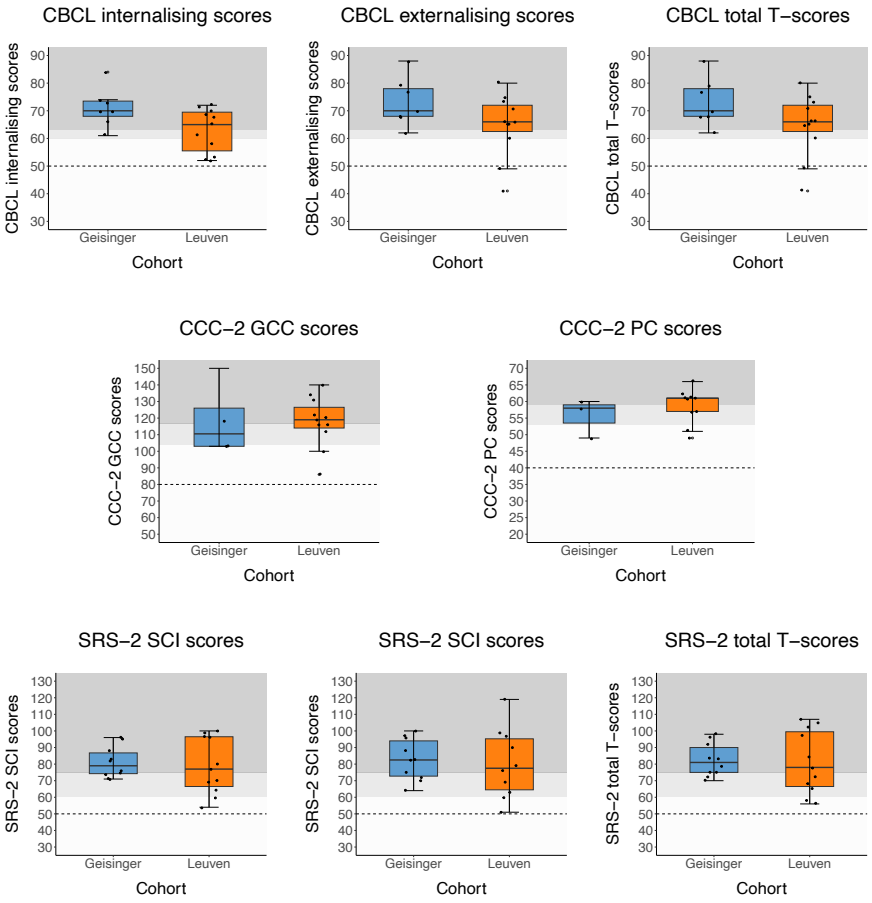
	16p11.2DS			**16p11.2Dup**		
	N	Pearson's *r*	*p*-value	*N*	Pearson's *r*	*p*-value
CBCL total – CCC-2 GCC	37	0.686	<0.001*	14	0.786	<0.001*
CBCL total – SRS-2 RIB	41	0.683	<0.001*	18	0.624	0.006*
CBCL total – FSIQ	33	-0.220	0.219	15	-0.070	0.803
CCC-2 GCC – SRS-2 RIB	37	0.611	<0.001*	15	0.677	0.006*
CCC-2 GCC – FSIQ	32	-0.508	0.003*	14	-0.510	0.063
SRS-2 RIB – FSIQ	35	-0.291	0.089	17	-0.535	0.027

Note. Statistical outcomes: *p*-value; *significant after Bonferroni correction at $p < 0.008$. GCC, general communication composite; RIB, Restricted Interests and Repetitive Behaviour.



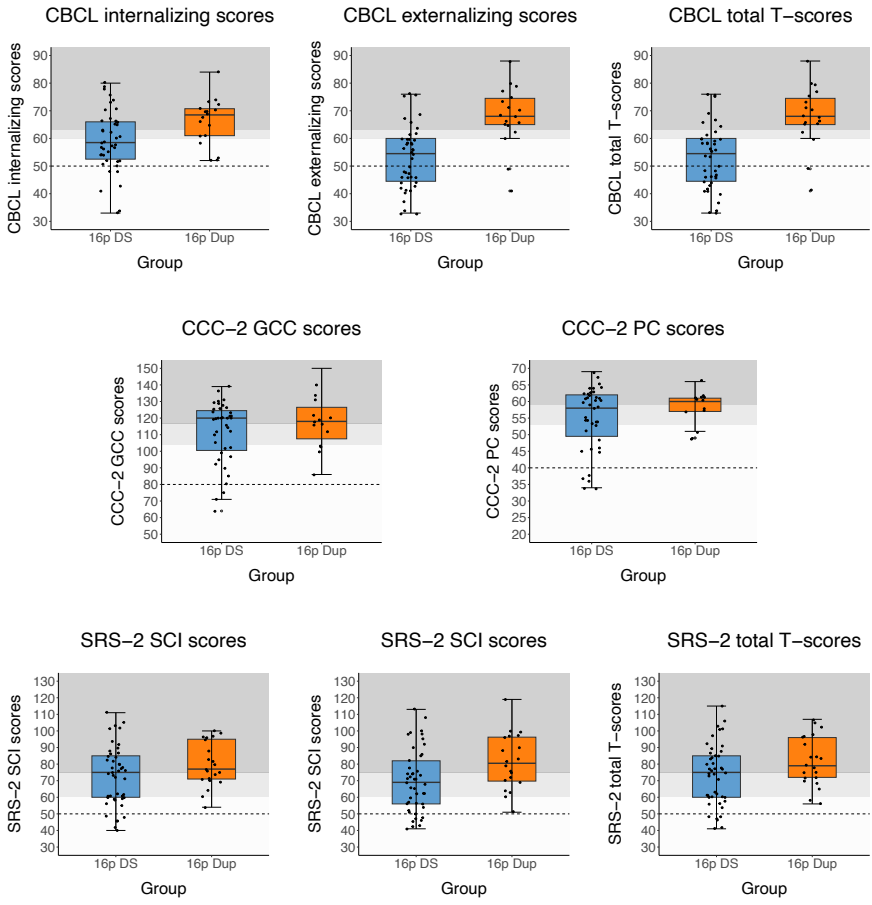
Supplementary Figure 5.1A – Boxplots summary scores in 16p11.2DS across cohort sites.

The dashed lines display norm group averages. The grey zones indicate the severity of the problems; the darker the grey, the more severe the difficulties: mild-moderate = light grey zone and severe = darker grey zone, based on clinical cut-off scores for CBCL, CCC-2 and SRS-2. Abbreviations. CBCL (norm group average = 50, cut-off: >59 = borderline, ≥64 = clinical); GCC, general communication composite (norm group average = 80, cut-off: >104 = mild-moderate, ≥117 = severe); PC, pragmatic composite (norm group average = 40, cut-off: >53 = mild-moderate, ≥60 = severe); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour (norm group average = 50, cut-off: >60 = mild–moderate, ≥76 severe).

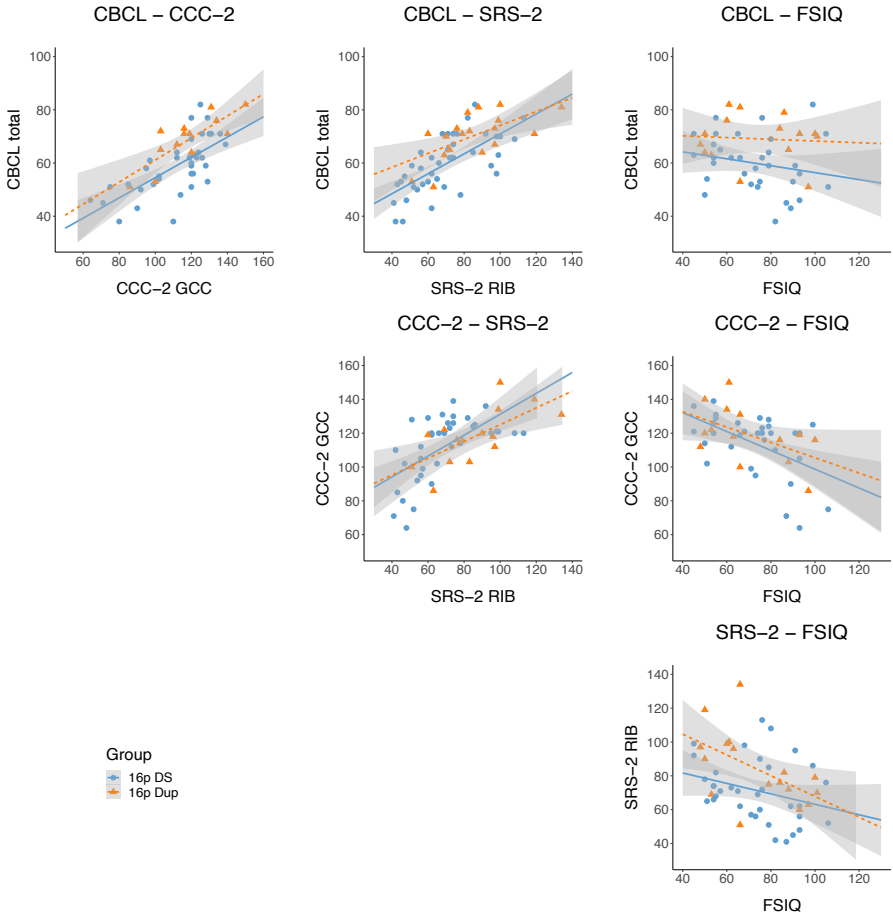


Supplementary Figure 5.1B - Boxplots summary scores in 16p11.2Dup across cohort sites.

The dashed lines display norm group averages. The grey zones indicate the severity of the problems; the darker the grey, the more severe the difficulties: mild-moderate = light grey zone and severe = darker grey zone, based on clinical cut-off scores for CBCL, CCC-2 and SRS-2. Abbreviations. CBCL (norm group average = 50, cut-off: >59 = borderline, ≥64 = clinical); GCC, general communication composite (norm group average = 80, cut-off: >104 = mild-moderate, ≥117 = severe); PC, pragmatic composite (norm group average = 40, cut-off: >53 = mild-moderate, ≥60 = severe); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour (norm group average = 50, cut-off: >60 = mild–moderate, ≥76 severe).



Supplementary Figure 5.2 – Boxplots summary scores across 16p11.2 CNVs.
The dashed lines display norm group averages. The grey zones indicate the severity of the problems; the darker the grey, the more severe the difficulties: mild-moderate = light grey zone and severe = darker grey zone, based on clinical cut-off scores for CBCL, CCC-2 and SRS-2. Abbreviations. CBCL (norm group average = 50, cut-off: >59 = borderline, ≥64 = clinical); GCC, general communication composite (norm group average = 80, cut-off: >104 = mild-moderate, ≥117 = severe); PC, pragmatic composite (norm group average = 40, cut-off: >53 = mild-moderate, ≥60 = severe); SCI, Social Communication and Interaction, RIB, Restricted Interests and Repetitive Behaviour (norm group average = 50, cut-off: >60 = mild–moderate, ≥76 severe).



Supplementary Figure 5.3 – Scatterplots correlations.
Abbreviations. GCC, general communication composite; RIB, Restricted Interests and Repetitive Behaviour; FSIQ, Full-Scale Intelligence Quotient

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Chapter 6

Chapter 6 - Developmental milestones and cognitive trajectories in school-aged children with 16p11.2 deletion

The content of this chapter is under revision for *Journal of Neurodevelopmental Disorders* as: Verbesselt, J., Breckpot, J., Zink, I., & Swillen, A. (2023). Developmental milestones and cognitive trajectories in school-aged children with 16p11.2 deletion. Journal of Neurodevelopmental Disorders, under revision. Supplementary material is provided at the end of this chapter.

Abstract

Background: 16p11.2 deletion syndrome (16p11.2DS) is a recurrent CNV that occurs *de novo* in approximately 70% of cases and confers risk for neurodevelopmental disorders, including intellectual disability (ID) and autism spectrum disorders (ASD). The current study focusses on developmental milestones, cognitive profiles and longitudinal cognitive trajectories.

Methods: In-person assessments, digital medical records and parental interviews on developmental history of 24 children (5-16 years) with a confirmed BP4-BP5 16p11.2DS were reviewed and analysed for developmental milestones (motor, language, continence). Standardised intelligence tests were administered in all children, and longitudinal IQ-data were available for a subgroup (79%, 19/24).

Results: Motor, language, and continence milestones were delayed. Average IQ was in the borderline range (IQ 71) with 46% (11/24) having borderline IQ (IQ 70-84). Both intra- and interindividual variability were found across the five cognitive domains with significant discrepancies between verbal and non-verbal skills in 55% (11/20). Longitudinal IQ-data indicate that school-aged children with 16p11.2DS perform statistically significantly lower at the second time point ($p < 0.001$) with 58% showing a growing into deficit trajectory.

Conclusion: Delayed motor, language and continence milestones are common in 16p11.2DS carriers. School-aged children with 16p11.2DS show increasing cognitive impairments over time, pointing to the need for early diagnosis, regular cognitive follow-up and individualised intervention. The high

prevalence of disharmonic IQ-profiles highlights the importance of expanding the focus beyond full-scale IQ (FSIQ) outcomes. Future studies in larger cohorts including carrier relatives are needed to gain more insight into the penetrance and phenotypic variability of 16p11.2DS.

6.1 Introduction

The proximal 16p11.2 deletion syndrome (16p11.2DS) defined by breakpoints 4 and 5, and encompassing 29 genes, is one of the most frequent copy number variants (CNVs) in the general population (Goldenberg, 2018; Jacquemont et al., 2011; Walters et al., 2010). The deletion occurs *de novo* in approximately 70% of cases (Niarchou et al., 2019). While existing literature outlines a spectrum of clinical manifestations, including reduced penetrance and variable expressivity, several gaps persist in our understanding of the neurodevelopmental and cognitive aspects and trajectories of 16p11.2DS.

Previous research has focused on clinical features associated with the deletion, such as overweight/obesity and seizures, and neurodevelopmental features, including psychiatric issues, speech-language and motor impairments, and autism spectrum disorders (ASD) (Chawner et al., 2019; Chung et al., 2021; D'Angelo et al., 2016; Goldman et al., 2019; Hanson et al., 2015; Jacquemont et al., 2011; Kim et al., 2020; Mei et al., 2018; Niarchou et al., 2019; Steinman et al., 2016; Taylor et al., 2021; Zufferey et al., 2012). Despite consistent reports of developmental delays in 16p11.2DS, exact data on motor and language milestones and time of bladder control have not been characterised before. In addition, the question raises whether the time point of reaching developmental milestones could be informative of later cognitive functioning.

Cognitive capacities exhibit a broad range from average IQ to intellectual disability (ID) with average full-scale IQ (FSIQ) falling within the borderline range (IQ 70-84) (Chawner et al., 2021; Hanson et al., 2015; Jutla et al., 2020; Kim et al., 2020; Mei et al., 2018; Modenato et al., 2021; Moreno-De-Luca et al., 2015). Verbal and non-verbal IQ (VIQ – NVIQ) scores are within the same range, with on average, slightly higher NVIQ scores (Chawner et al., 2021; D'Angelo et al., 2016; Hanson et al., 2015; Jacquemont et al., 2022; Moreno-De-Luca et al., 2015; Mortillo & Mulle, 2021). In one study, VIQ was significantly lower than NVIQ (Zufferey et al., 2012). Moreover, a trend towards lower FSIQ in patients with inherited 16p11.2DS (FSIQ 74) was found compared to patients with *de novo* deletions (FSIQ 83), which aligns with findings from other studies (D'angelo et al., 2016; Gill et al., 2014; Hanson et al., 2015). While previous

studies have predominantly reported on the overall cognitive outcomes and the rather limited and outdated VIQ-NVIQ comparisons, detailed cognitive profiles based on primary index scores (WISC-V) remain unexplored. Additionally, longitudinal studies beyond the age of seven are lacking in the 16p11.2 population (Bernier et al., 2017). Consequently, it remains unknown how their cognitive skills continue to develop during primary and secondary school. Understanding cognitive profiles and trajectories associated with 16p11.2 CNVs has scientific value for advancing our understanding of genotype-phenotype correlations, while holding clinical importance in setting clear expectations for families and facilitating treatment planning and monitoring (Bernier et al., 2017).

This study aims to address these gaps by comprehensively characterising the developmental phenotype of school-aged children with 16p11.2DS. The objective is to investigate early developmental milestones, cross-sectional cognitive profiles, and longitudinal cognitive trajectories within this population, while exploring the potential association between the attainment of developmental milestones and intelligence outcomes. Furthermore, we want to delineate the broad cognitive profiles based on the WISC-V cognitive indices to look for potential cognitive signatures of the 16p11.2DS and compare these to the existing literature based on VIQ-NVIQ comparisons.

6.2 Methodology

6.2.1 Participants

In total, 24 school-aged children between 5-16 years (median age = 10.8 years) participated in this study. Following a genetics first approach, all children had a confirmed genetic diagnosis of 16p11.2DS defined by breakpoints 4 and 5 (BP4-BP5) by microarray. Exclusion criteria included extreme prematurity (<32 weeks) and the presence of additional (likely) pathogenic chromosomal variants. Patient characteristics are summarised in Table 6.1. All patients were index patients, who were referred to the centre of Human Genetics at University Hospitals Leuven in Belgium based on developmental delays (42%, 10/24), medical concerns (4%, 1/24) or a combination of both (54%, 13/24). The deletion occurred *de novo* in the majority of patients (81%, 13/16), while it was inherited in 19% (3/16).

Table 6.1 – Sociodemographic characteristics, educational and genetic data in 16p11.2DS (*n* = 24)

16p11.2DS	
Sample Size (*n*)	24
Sex (*n*, %)	
Male	10/24 (42%)
Female	14/24 (58%)
Chronological age (yrs.mo)	
Average (*SD*)	10.11 (3.3)
Median	10.8
Range	5.9 – 16.11
Inheritance pattern (*n*, %)	
De novo	13/24 (54%)
Inherited:	3/24 (13%)
- Maternally inherited	2/3 (67%)
- Paternally inherited	1/3 (33%)
Unknown*	8/24 (33%)
Socioeconomic status (*n*, %)**	
High	10/24 (42%)
Middle	12/24 (50%)
Low	2/24 (8%)
Indication for diagnosis	
Medical	1/24 (4%)
Developmental	10/24 (42%)
Medical + developmental	13/24 (54%)
Type of education (*n*, %)	
Special education	20/24 (83%)
Regular education	1/24 (4%)
Regular with assistance	3/24 (13%)
Therapy (n, %)	23/24 (96%)
Physiotherapy	18/24 (75%)
Speech-language therapy	19/24 (79%)
Occupational therapy	7/24 (29%)
Psychotherapy	3/24 (13%)
Home-based early intervention	8/24 (33%)
Formal diagnosis of autism spectrum disorder	11/23 (48%)***
IQ range (*n*, %)	
<55	4/24 (17%)
55-70	5/24 (21%)
71-85	11/24 (46%)

86-100	4/24 (17%)

Note. *foster care (n = 2), maternal inheritance ruled out, parents declined genetic testing. **The educational level achieved by the (foster) mother was utilised as a substitute measure to assess socioeconomic status (SES). The categorisation of SES relied on the International Standard Classification of Education (ISCED) provided by UNESCO (OECD, 2017; UNESCO Institute for Statistics., 2012). The classification involved three main categories: low (primary education or lower high school grades), middle (secondary/high school education), and high (Bachelor's, Master's, or Doctoral Degrees). *** For one child, no data were available on formal diagnosis of autism spectrum disorder.

6.2.2 Procedures and measures

Patients were invited to the clinic or seen during home visits to collect data in a prospective way. Information about developmental milestones, referring to the timely achievement of milestones across several developmental areas (motor, language, continence), was gathered through clinical follow-ups or parental anamnestic reports. Data from digital medical records, in-person assessments and parental interviews were reviewed and analysed.

Following a standardised research protocol, the most recent Dutch version of the Wechsler Intelligence Scale for Children – Fifth Edition (WISC-V-NL; Hendriks et al., 2019; Wechsler, 2014) was administered in all, unless an intelligence test had been administered in the past year. This was the case for three children who had been tested with the Dutch versions of the Wechsler Preschool and Primary Scale of Intelligence – Fourth Edition (WPPSI-IV-NL; Wechsler, 2012), Snijders–Oomen Nonverbal test Revised (SON-R; Tellegen & Laros, 2017) and Wechsler Nonverbal Scale of Ability (WNV-NL; Wechsler & Naglieri, 2008). Using age-referenced norm tables, a Full-Scale IQ (FSIQ), five Primary Index Scales (i.e., Verbal Comprehension (VCI), Visual Spatial (VSI), Fluid Reasoning (FRI), Working Memory (WMI), and Processing Speed (PSI)) and one Ancillary Index Scale (Nonverbal (NVI)) were computed (M = 100, SD = 15) for all patients. However, due to three participants being insufficiently verbal, only NVI scores could be derived and utilised for the analyses.

In the majority (79%, 19/24) of patients, IQ scores were available at two or three different time points, providing insights into longitudinal cognitive trajectories. The initial evaluation involved the Dutch edition of Bayley Scales of Infant Development - Second edition (BSID-II-NL; Bayley, 1993; van der Meulen et al., 2004), resulting in a developmental quotient (DQ), whereas for the subsequent evaluation either the Wechsler Preschool and Primary Scale of Intelligence – Fourth Edition (WPPSI-III-NL; Wechsler, 2002; Wechsler et al.,

2009), WPPSI-IV-NL, Wechsler Intelligence Scale for Children – Third Edition (WISC-III-NL; Wechsler, 1991), SON-R or WISC-V were utilised. The most recent IQ assessment was part of the current research protocol, which consisted of the WISC-V (n = 18) or the SON-R (n = 1), as described above. Intelligence scores were compared across the different time points to get insight in the longitudinal cognitive trajectories. If the non-verbal intelligence test SON-R was administered, only NVI scores were compared across time points.

6.2.3 Statistical analyses

One sample t-tests were executed to explore potential age differences regarding the attainment of early motor and language milestones in the 16p11.2DS group compared to the general population, whereas Binomial tests were used to compare the proportions of participants who achieved bladder and bowel control at five years of age to the proportions in the general population. One sample t-tests were also performed to compare IQ scores in the 16p11.2DS group to those of the general population. Bonferroni correction was applied to correct for multiple testing. One-way ANOVA was run to compare the means of the five WISC-V Primary Index Scales. Pearson product-moment correlation analyses were run to investigate the relation between the achievement of early developmental milestones and IQ outcomes. Paired Samples t-tests were performed to compare IQ outcomes between the first and second, and second and third time point. Cohen's d was used as effect size parameter with values of ≥ 0.2, ≥ 0.5, ≥ 0.8 interpreted as small, moderate or large effects respectively. Linear regression was performed with age as covariate to determine an influence of chronological age on intelligence measures. Statistical analyses were run using JASP (JASP Team, 2022) and R (R core team, 2017; Wickham, 2016).

6.3 Results

6.3.1 Attainment of developmental milestones in children with 16p11.2DS

Figure 6.1 depicts the proportion of children attaining developmental milestones across several domains as a function of age, reflecting a wide age range. The dotted lines represent the median age of milestone achievement in the 16p11.2DS group. Children with 16p11.2DS first walked independently at a median age of 17.5 months, while their first words and simple phrases were spoken at a median age of 18 and 30 months respectively. None of the children with 16p11.2DS could walk independently at 12 months, which is the mean age

in the typical population (WHO Multicentre Growth Reference Study Group., 2006), and only one of the children used a single word by that age (Conti-Ramsden & Durkin, 2012; Sheldrick & Perrin, 2013; Visser-Bochane et al., 2020). Twenty-six percent (6/23) formed simple phrases by combining two words at the mean age of the normative population (24 months). Daytime bladder control and bowel control were attained at median ages of 33 and 36 months respectively, whereas night-time bladder control was achieved at a median age of 4 years (48 months).

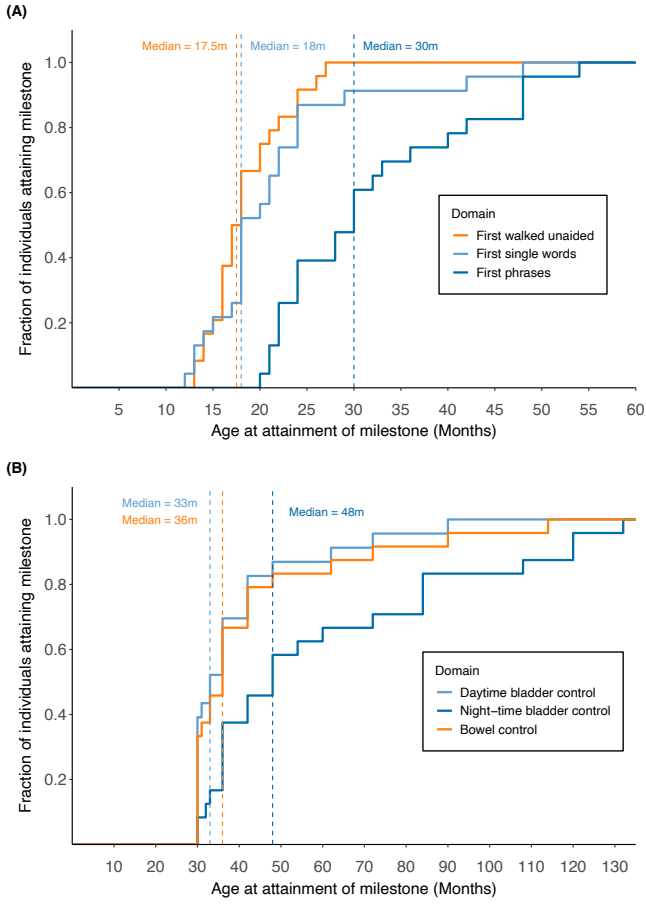


Figure 6.1 – Developmental trajectory of participants with 16p11.2DS.
(A) Age at attainment of gross motor and language milestones. The solid lines indicate the time course of achievement of walking independently or speaking the first words/phrases by our cohort. (B) Age at attainment of continence milestones. The solid lines indicate the time course of achievement of day- and night-time bladder and bowel control by our cohort. The dashed lines show the median age at the attainment of these milestones in our cohort.

Table 6.2 provides the descriptive statistics of the attainment of developmental milestones in children with 16p11.2DS. One sample Wilcoxon-signed rank *t*-tests were used to compare the milestones to these of typically developing children in the general population. The results show statistically significant delays in the attainment of both language and motor milestones among children with 16p11.2DS, with large effect sizes. Furthermore, employing binomial tests, we found no statistically significant difference in the proportion of children with diurnal enuresis compared to the general population (13%/17% vs. 7%). However, a significantly higher proportion of individuals with 16p11.2DS experienced nocturnal enuresis at age 5 (60 months) compared to the proportion in the general population (38% vs. 17%).

Table 6.2 – Developmental milestones and FSIQ in 16p11.2DS (n = 24) compared to the general population

Developmental milestones and FSIQ	**16p11.2DS** *N* *M (SD)* Median Range	**Statistical outcomes group level** One sample *t*-test (*t*/*V* = , *p* = ,*d*/ *r* =) or Binomial test (*p* =)
First walked unaided (months)		
Average (*SD*)	18 (4)	*V* = 300.000
Median	17.5	*p* < 0.001**
Range	13 – 27	*r* = 1.000
Motor delays (*n*, %)	16/24 (67%)	
Age of first single words (months)		
Average (*SD*)	21 (9)	*V* = 253.000
Median	18	*p* < 0.001**
Range	12 – 48	*r* = 1.000
Age of first single phrases (months)		
Average (*SD*)	32 (10)	*V* = 188.000
Median	30	*p* = 0.002**
Range	20 – 54	*r* = 0.790
Speech-language delays (*n*, %)	22/24 (92%)	
Age at daytime bladder control (months)		
Average (*SD*)	40 (15)	
Median	33	
Range	30 – 90	
Bladder control delays (*n, %*)	3/24 (13%)	*p* = 0.215

Age at night-time bladder control (months)		
Average (*SD*)	60 (32)	
Median	48	
Range	30 – 132	
Bladder control delays (*n, %*)	9/24 (38%)	$p = 0.013$**
Age of bowel control (months)		
Average (*SD*)	43 (21)	
Median	36	
Range	30 – 114	
Bowel control delays (*n, %*)	4/24 (17%)	$p = 0.083$
FSIQ		
Average (*SD*)	71 (13)	$t = -10.466$
Median	74	$p < 0.001$**
Range	45 – 91	$d = -2.284$
Intellectual disability (*n, %*)	9/24 (38%)	

Note. *significant at $p < 0.05$, **significant after Bonferroni correction at $p < 0.016$. Abbreviations: IQ, intellectual quotient; *SD*, standard deviation. First walked unaided/first single words (norm group average = 12 months, cut-off > 18 months = delayed), First single phrases (norm group average = 24 months, > 30 months = delayed), Daytime bladder/bowel control (no control in 7% at 60 months in norm group), night-time bladder control (no control in 17% at 60 months in norm group), FSIQ (norm group average = 100, cut-off < 70 = intellectual disability) (Conti-Ramsden & Durkin, 2012; Kliegman et al., 2015; Nieuwhof-Leppink et al., 2019; Sheldrick & Perrin, 2013; Visser-Bochane et al., 2020; WHO Multicentre Growth Reference Study Group., 2006).

6.3.2 Intellectual, learning profiles and influencing factors

The average FSIQ in our cohort at the most recent timepoint is 71, with 38% exhibiting mild-to-moderate ID. Figure 6.2 illustrates that the gaussian curve of the 16p11.2DS group has shifted approximately 30 IQ points (≈1.93 *SD*) to the left, in comparison to the distribution of the general population ($M = 100$, $SD = 15$). One sample *t*-tests reported in Table 6.2 confirmed statistically significantly lower FSIQ scores in school-aged children with 16p11.2DS compared to their typically developing peers in the general population, with a large effect size. As shown in Table 6.1, 96% of children with 16p11.2DS (23/24) received therapy, referring predominantly to speech-language therapy (19/24, 79%) and physiotherapy (18/24, 75%). Regarding education, 83% (20/24) attended special education and only one child with 16p11.2DS followed regular education without assistance.

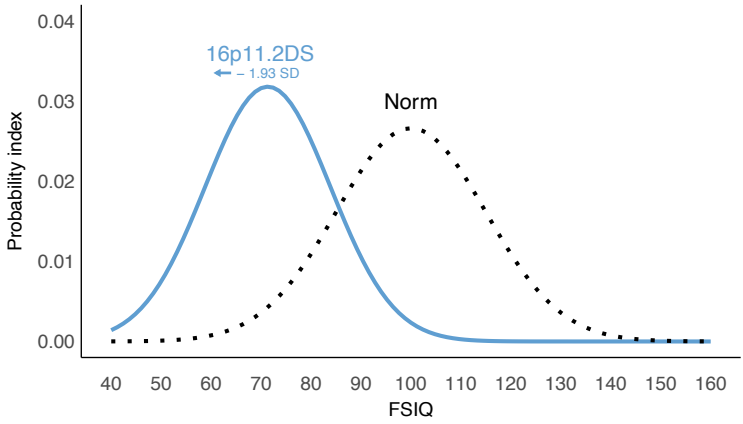


Figure 6.2 – Gaussian curve of FSIQ in 16p11.2DS (*n* = 21).
The dashed line depicts the normal distribution of FSIQ in the general population (*M* = 100, *SD* = 15). *SD* shifts are calculated in relation to the norm group sample.

Descriptive statistics and boxplots on potentially confounding factors, such as inheritance pattern, comorbid ASD or attention-deficit/hyperactivity disorder (ADHD), and sex, can be found in Supplementary Table 6.1 and Supplementary Figure 6.1. Qualitatively, FSIQ scores of children with *de novo* deletions (n = 10, M = 73) are 10 points higher than those of children with inherited deletions (n = 3, M = 63). Children with ASD (n = 9, M = 70) performed on average similar to children without ASD (n = 11, M = 72). Children with ADHD (n = 7, M = 66) obtained on average lower FSIQ scores compared to children without ADHD (n = 14, M = 74). In addition, FSIQ outcomes of boys (n = 8, M = 77) were on average 9 points higher than those of girls (n = 13, M = 68). Age did not have an influence on the FSIQ outcomes (p = 0.502).

Figure 6.3 displays boxplots of the IQ scores of the five WISC-V Primary Index Scales. The grey zones delineate the areas of borderline IQ (IQ 70-84) and mild-moderate ID (<70), whereas the dashed line depicts the norm group average (M = 100). The average scores of the five Primary Index Scales are in the borderline range (IQ 70-84), displaying a relatively wide variability, except for the Working Memory Index (Supplementary Table 6.2). Levene's test could not confirm statistically significantly different variances across these Index Scales ($F(4)$ = 2.039, p = 0.094). One-way ANOVA revealed no statistically significant differences across the five Primary Index Scales and Nonverbal Index ($F(5)$ = 1.287, p = 0.274, η^2 = 0.049).

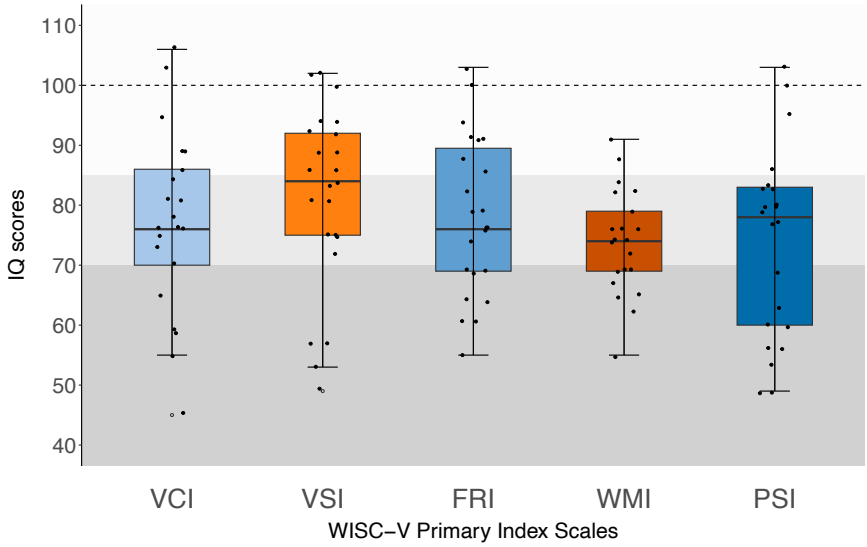


Figure 6.3 – Boxplots across WISC-V Primary Index Scales in 16p11.2DS.
The dashed line represents the norm group average (*M* = 100, *SD* = 15). The grey zones delineate borderline IQ (70-84) and mild-moderate IQ (<70). Abbreviations: VCI, Verbal Comprehension Index; VSI, Visual Spatial Index; FRI, Fluid Reasoning Index; WMI, Working Memory Index; PSI, Processing Speed Index.

Supplementary Figure 6.2 shows the individual Index profiles for all children with 16p11.2DS, demonstrating both intra- and interindividual variability across the five Primary Indices. Examining relative strengths and weaknesses within individuals according to the WISC-V manual revealed that the Visual Spatial index is the index for which most individuals exhibit a relative strength (35%, 7/20) and none a relative weakness. Using the WISC-V interpretive report, pairwise difference comparisons at the Primary Index level revealed that all individuals (19/19) demonstrated at least one (1/10) significant difference, with 79% (15/19) showing at least three (3/10) significant differences (Supplementary Table 6.2). To interpret the Index Scales results in the context of the existing literature, we also compared the Verbal Comprehension Index (VCI) and the Nonverbal Index (NVI) within individuals. Disharmonic VCI versus NVI profiles were identified as a difference of at least 15 IQ points (1 *SD*) between both indices (Graauwmans et al., 2017) and observed in 55% (11/20) of children, with 40% (8/20) showing significantly higher VCI, whereas 15% (3/20) demonstrated significantly higher NVI. More details on the Primary Index Scales can be found in Supplementary Table 6.3.

6.3.3 Association between developmental milestones and FSIQ scores in 16p11.2DS

Pearson correlation analyses were run to investigate the potential relation between the age at attainment of early developmental milestones across motor, language and continence domains and IQ scores in the 16p11.2DS population. Correlation plots can be found in Supplementary Figure 6.3. None of the developmental milestones were statistically significantly associated with the FSIQ outcomes in the 16p11.2DS group ($r < 0.271$, $0.235 > p > 0.913$). In addition, no significant correlations were found between the achievement of motor, language and continence milestones ($r < 0.370$, $0.082 > p > 0.977$).

6.3.4 Cognitive trajectories

A subgroup of individuals had formal IQ assessment at two or three time points. The comparison in the youngest group ($n = 11$) included a first evaluation by the BSID-II-NL in early toddlerhood at a median age of 30 months (2.6 years, range 1.3 – 3.2 years) and a second evaluation by the SON-R or WPPSI-III in preschool at a median age of 5.7 years (age range 3.7 – 6.9 years). The mean change in IQ score between these two measurements showed a 12-point increase in favour of the second assessment (Supplementary Figure 6.4). A Paired Samples *t*-test within participants revealed statistically significantly lower scores ($t(10) = -2.862$, $p = 0.017$, $d = -0.863$) at the first time point (average developmental quotient T1 = 71, range 55 – 91) than at the second time point (average IQ T2 = 83, range 50 – 105), with a large effect size.

The comparison in the oldest group ($n = 19$) consisted of a first evaluation by the SON-R, WPPSI-III or WISC-III at a median age of 5.10 years (age range 3.4 years – 10.1 years) and a second evaluation by the WISC-V or SON-R at a median age of 11.5 years (age range 5.9 – 16.11 years). The IQ scores at both time points are plotted in Figure 6.4. The dotted lines indicate the cognitive trajectory of each participant, revealing a downward trend towards the second time point. A Paired Samples *t*-test confirmed statistically significantly lower IQ scores with a large effect size at the second and most recent time point (average IQ T1 = 83, average IQ T2 = 71, $t(18) = -6.297$, $p < 0.001$, $d = -1.445$).

Forty-two percent (8/19) of children with 16p11.2DS demonstrated a relatively stable cognitive trajectory, whereas 58% (11/19) showed a growing into deficit trajectory, indicated by the difference of more than 10 IQ points between the two assessments. A relatively stable trajectory is distinguished by the progress

in raw scores on subtests, demonstrating sufficient development, while scaled and standard scores remain consistent over time. Individuals categorised as growing into deficit or experiencing a developmental lag are those who exhibit inadequate progress as they age, leading to an expanding gap in comparison to the general population. Their developmental pace is notably slower than that of their typically developing peers, resulting in reduced scaled and standard scores on specific subtests (Chawner et al., 2017; Duijff et al., 2012; Swillen & McDonald-McGinn, 2015; Van Den Heuvel et al., 2018; Verbesselt et al., 2022).

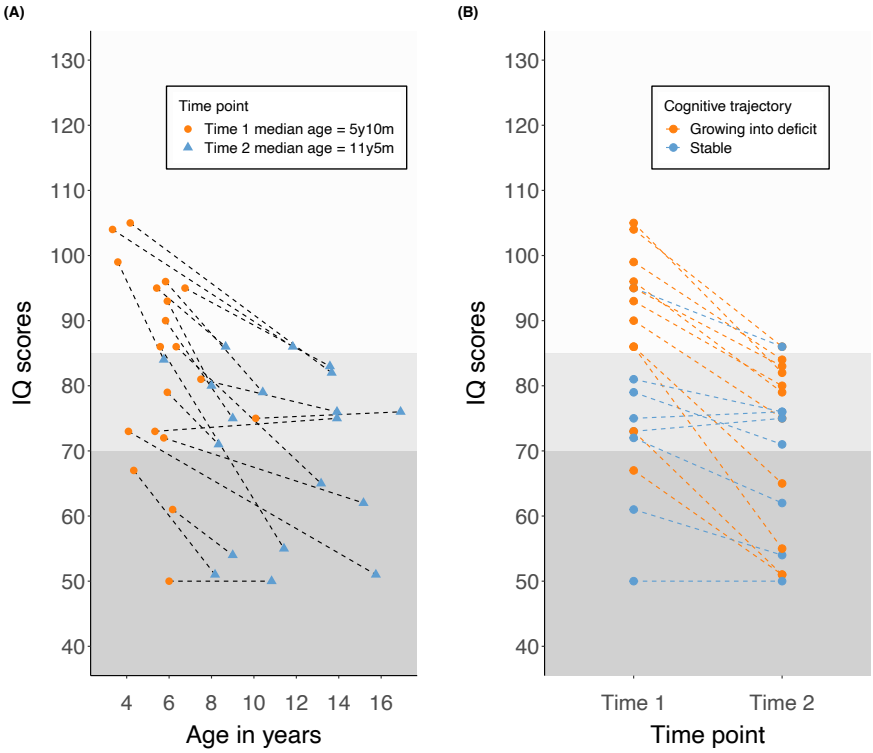


Figure 6.4 – Longitudinal cognitive trajectories in children with 16p11.2DS (*n*=19). (A) IQ scores as a function of age for each participant. The colour and shape refer to the time point. The dashed lines represent the individual cognitive trajectories. (B) IQ scores at two different time points (median age T1 5y10m, median age T2 11y5m). The colours refer to the cognitive trajectories: stable (|IQ T2 – T1| <10) and growing into deficit (IQ T1 – T2 > 10).

6.4 Discussion

The aim of the current study was to characterise developmental milestones, broad cognitive profiles/indices and cognitive trajectories of 24 school-aged children with the proximal BP4-BP5 16p11.2DS, using a standardised protocol that consisted of in-person formal cognitive assessments, parental interviews and reviewing digital medical records.

Regarding developmental milestones, results indicated that almost all children with 16p11.2DS (92%) experienced language delays, whereas 67% showed motor delays. Mean age of walking was at 18 months of age, which was similar to the mean age found by Zufferey et al. (2012). Compared to the general population, median ages of achieving motor and language milestones were significantly delayed. In addition, significantly more children with 16p11.2DS experienced nocturnal enuresis at 60 months of age. In general, incontinence is more often reported in children with genetic syndromes and/or neurodevelopmental disorders such as ID and autism spectrum disorders (Matson & Kozlowski, 2011; von Gontard et al., 2022). Moreover, within these syndromes, the prevalence of incontinence increases in children with more severe levels of ID. However, in the current study, we did not find any significant associations between bladder or bowel control and IQ. Possible factors that might play a role in the delayed age of reaching night-time bladder control in genetic populations could be the presence of epilepsy and/or motor problems (von Gontard et al., 2022), which are common in 16p11.2DS, but this warrants further research. Another older study in a large sample (n = 1666) of typically developing children concluded that nocturnal enuresis might be associated with motor and language milestones (Touchette et al., 2005). However, we could not confirm these associations in the present study, which might be partially due to the much smaller sample.

The average FSIQ of children with 16p11.2DS in the current study is 71, whereas other studies have reported average FSIQ scores ranging from 69 to 92 (Chawner et al., 2021; D'Angelo et al., 2016; Hanson et al., 2015; Jutla et al., 2020; Kim et al., 2020; Maillard et al., 2016; Mei et al., 2018; Modenato et al., 2021; Moreno-De-Luca et al., 2015). Consequently, the current cohort is situated at the lower end of the IQ-spectrum, which might be partially explained by methodological differences, such as used test instruments, age differences, and distinct ascertainment strategies dependent on the clinical setting. In addition, 38% of our cohort showed mild-to-moderate ID, aligning with findings by

Niarchou et al. (2019), who reported ID in a similar proportion (30%) of patients. Qualitatively, children with *de novo* 16p11.2DS achieved higher FSIQ compared to those with inherited 16p11.2DS, consistent with previous research (D'angelo et al., 2016; Gill et al., 2014; Hanson et al., 2015; Zufferey et al., 2012). It is noteworthy that FSIQ scores did not exhibit large or clinically relevant differences among children with comorbid neurodevelopmental disorders, neither were these scores dependent on sex or age. However, these preliminary, descriptive findings should be validated in a larger sample. Most children with 16p11.2DS received therapy during childhood, including physiotherapy and speech-language therapy, which was also reported by Zufferey et al. (2012). Aligning with their overall intellectual capacities, most children attended special education, which points to the need for individualised educational support.

To the best of our knowledge, the current study is the first to examine the five WISC-V Primary Index Scales, revealing both intra- and interindividual variability across the 5 cognitive indices. All mean Index IQ scores (Verbal Comprehension, Perceptual Reasoning, Fluid Reasoning, Working Memory and Processing Speed) fall within the borderline range, displaying considerable heterogeneity, except for the Working Memory Index. The substantial diversity among individuals highlights the significance to not only focus on group averages but also to carefully assess the performance of the individual child. Visual spatial skills were found to be a relative strength in one-third of the children. Apart from that, there were no particularly distinctive cognitive characteristics that prominently stood out within the 16p11.2DS group. Disharmonic VCI versus NVI profiles, defined by a discrepancy of at least 15 IQ points between verbal and nonverbal skills, were found in 55% (11/20) of children, with the majority (8/11) exhibiting better verbal than nonverbal skills. The large variability within individuals points to the importance of looking beyond FSIQ outcomes. Analysing the different Index Scales of the WISC V enables us to gain a comprehensive understanding of an individual's broader cognitive capacities, and it allows us to tailor interventions accordingly to the specific strengths and weaknesses.

At the group level, children with 16p11.2DS demonstrated, on average, slightly better verbal (VCI) than nonverbal skills (NVI). This finding contrasts with previous studies that suggested a greater impact on verbal than nonverbal skills (Chawner et al., 2021; D'Angelo et al., 2016; Hanson et al., 2015; Jacquemont et al., 2022; Kim et al., 2020; Moreno-De-Luca et al., 2015; Mortillo & Mulle, 2021; Owen et al., 2018), with significant differences only observed in

Zufferey et al. (2012). However, these studies were predominantly based on the same cohorts, in particular the 16p11.2 European and Simons Variation in Individuals Project (Simons VIP) consortia and the Cardiff University Experiences of Children with Copy Number Variants (ECHO) study. In the current study, at the group level, no statistically significant differences were found across the different Index Scales. Only Bernier et al. (2017) reported, on average, higher verbal skills from age 5 onward, aligning more closely with the current results. Reported differences might be partially related to the use of different IQ-measurements and subtests to assess verbal and nonverbal skills. Future studies should further investigate these Index Scales to fully delineate the broad intellectual and cognitive profile of children with 16p11.2DS.

Longitudinal cognitive trajectories in the youngest group revealed a significant 12-point increase from the first assessment in early toddlerhood to the second assessment in preschool, partially corresponding to the results from Bernier et al. (2017), who observed improvements in verbal IQ from 2 to 7 years of age. However, it is essential to acknowledge the limitations of the developmental quotient (DQ) derived from the BSID-II. One study reported that the DQ of the BSID-II is an insufficient predictor of later IQ in typically developing children (Månsson et al., 2019). Furthermore, it is important to recognise that the DQ of BSID-II is not entirely equivalent to an IQ-score, as it encompasses cognition, language and motor skills. Since language and motor delays are common in the current group of children, these factors might have contributed to the initially overall lower DQ in early toddlerhood. In addition, many children in this study received speech-language or physiotherapy during early toddlerhood, suggesting that the observed increase towards the second assessment may, in part, reflect positive responses to therapeutic interventions. An alternative hypothesis is that at this early stage, predominantly children with more pronounced developmental delays are referred for formal DQ assessment, and therefore primarily represent the more severe end of the spectrum. Given the small sample size, the current results should be interpreted with caution.

Longitudinal cognitive trajectories in the oldest age group revealed a significant 13-point decrease from the first assessment in preschool to the second assessment in primary or the beginning of secondary school. Growing into deficit-trajectories were identified in 58% (11/19) of children. These results should be approached with some caution, due to the limited sample size, the use of different IQ measurements, and partially overlapping age ranges at the two time points. Nevertheless, growing into deficit-profiles have also been observed

in other recurrent CNV populations, such as 22q11.2 deletion syndrome or 22q11.2 duplication (Chawner et al., 2017; Duijff et al., 2012; Swillen & McDonald-McGinn, 2015; Van Den Heuvel et al., 2018; Verbesselt et al., 2022). These profiles might be partly accounted for by the rising emphasis on abstract reasoning skills in IQ measurements as children age, which could represent a relative weakness in 16p11.2DS. These longitudinal cognitive trajectories underscore the importance of regular cognitive (follow-up) assessments using standardised measures to provide individualised and adapted/adequate support as early as possible, since challenges may change at different ages and life stages.

6.4.1 Strengths, limitations and future

A key strength of the current study is the use of a standardised protocol for the prospective collection of cognitive and behavioural data, incorporating gold standard test instruments to assess IQ in person. This robust methodology is complemented by data collected from digital medical records and parental interviews on developmental history. Additionally, the rather narrow age range contributes to a clearer understanding of the developmental and cognitive phenotype among primary and early secondary school-aged children with 16p11.2DS.

Despite these strengths, certain limitations should be acknowledged. The relatively small sample size and the potential ascertainment bias within the current cohort prevent us from drawing generalised conclusions. Future studies in larger cohorts both from clinical and research settings, including carrier relatives, are needed to gain more insight into the penetrance and phenotypic variability of 16p11.2DS. Moreover, the use of different IQ tests, different ages at previous IQ assessments and the lack of more detailed data on the index scores poses a challenge for cross-time point comparisons. Longitudinal studies should be performed with the same IQ tests to assess IQ consistently at different time points and extend into adulthood. Additionally, future research should preferably include assessments of adaptive skills, since these are crucial for our understanding of the cognitive and functional outcomes in individuals with 16p11.2DS.

6.5 Conclusion

The present study aimed to elucidate developmental milestones, cross-sectional cognitive profiles and longitudinal cognitive trajectories of school-aged children with the proximal BP4-BP5 16p11.2DS. Our findings reveal a high prevalence of delayed motor, language and night-time bladder control milestones in individuals with 16p11.2DS, independent of their later cognitive outcomes. This is an important clinical observation that paediatricians and other healthcare professionals should be aware of. Children in early toddlerhood already demonstrate diverse cognitive profiles, with a subgroup undergoing formal IQ assessment due to pronounced developmental, motor and language delays. Most of them show positive responses to therapy, demonstrating the importance of early interventions. School-aged children with 16p11.2DS show increasing cognitive impairments with age, underscoring the need for regular cognitive assessments, follow-up and personalised educational intervention strategies. The large intra-individual variability shown by the high proportion of disharmonic cognitive profiles emphasises the need not only to look at FSIQ but also at the complete profile of cognitive domains including Verbal Comprehension, Perceptual Reasoning, Fluid Reasoning, Working Memory and Processing Speed. Finally, to fully capture and deepen our understanding of the penetrance and phenotypic variability of development and cognitive outcomes in 16p11.2DS, future studies should encompass larger cohorts, including carrier relatives.

6.6 Supplementary material

Supplementary Table 6.1 – Descriptive statistics WISC-V FSIQ scores across subgroups based on potential confounding factors

Inheritance pattern	*De novo*		
		N	3
		Average (*SD*)	63 (11)
		Median	68
		Range	51 – 71
	Inherited		
		N	10
		Average (*SD*)	73 (13)
		Median	76
		Range	45 – 89
ASD diagnosis	ASD		
		N	9
		Average (*SD*)	70 (11)
		Median	74
		Range	51 – 83
	No ASD		
		N	11
		Average (*SD*)	72 (15)
		Median	73
		Range	45 – 91
ADHD diagnosis	ADHD		
		N	7
		Average (*SD*)	66 (15)
		Median	65
		Range	45 – 86
	No ADHD		
		N	14
		Average (*SD*)	74 (11)
		Median	76
		Range	54 – 91
Sex	Female		
		N	13
		Average (*SD*)	68 (14)
		Median	71
		Range	45 – 89

	Male	
	N	8
	Average (*SD*)	77 (9)
	Median	75
	Range	65 – 91

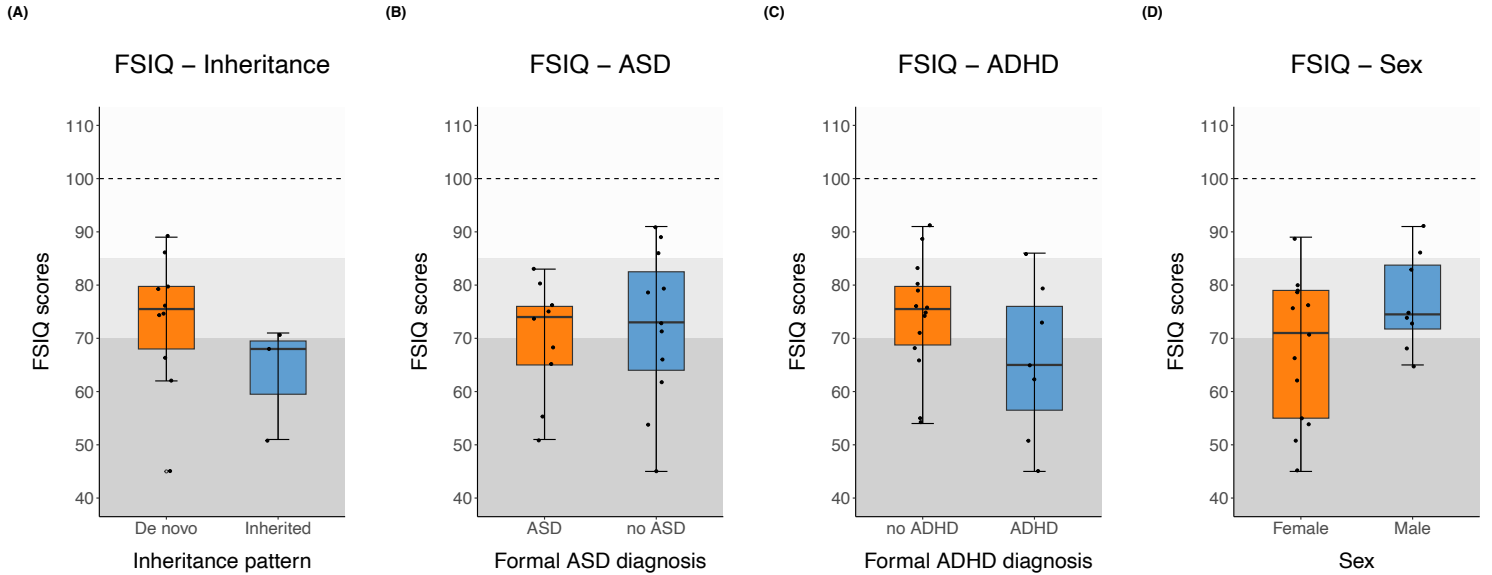
Supplementary Table 6.2 – Counts of Index level pairwise difference comparisons

Counts of Index level pairwise difference comparisons (out of 10)	**Frequency**	**Proportion**
1 significant pairwise difference	3/19	16%
2 significant pairwise differences	1/19	5%
3 significant pairwise differences	3/19	16%
4 significant pairwise differences	2/19	10%
5 significant pairwise differences	6/19	32%
6 significant pairwise differences	3/19	16%
7 significant pairwise differences	1/19	5%

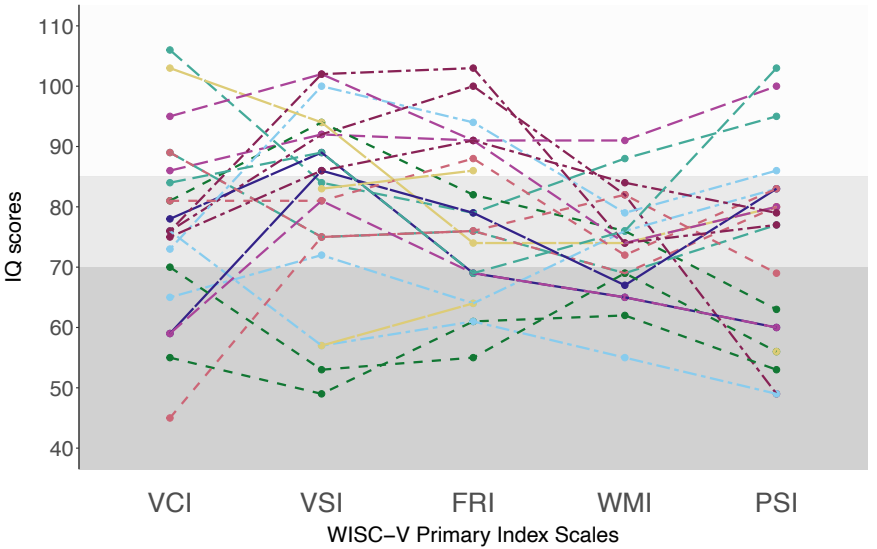
Supplementary Table 6.3 – Descriptive statistics WISC-V Primary Index Scales and one Ancillary Index Scale

WISC-V five Primary Index Scales	Verbal Comprehension Index (VCI)	
	N	21
	Average (*SD*)	77 (15)
	Median	76
	Range	45 – 106
	Visual Spatial Index (VSI)	
	N	23
	Average (*SD*)	81 (15)
	Median	84
	Range	49 – 102
	Fluid Reasoning Index (FRI)	
	N	23
	Average (*SD*)	78 (13)
	Median	76
	Range	55 – 103
	Working Memory Index (WMI)	
	N	21
	Average (*SD*)	74 (9)

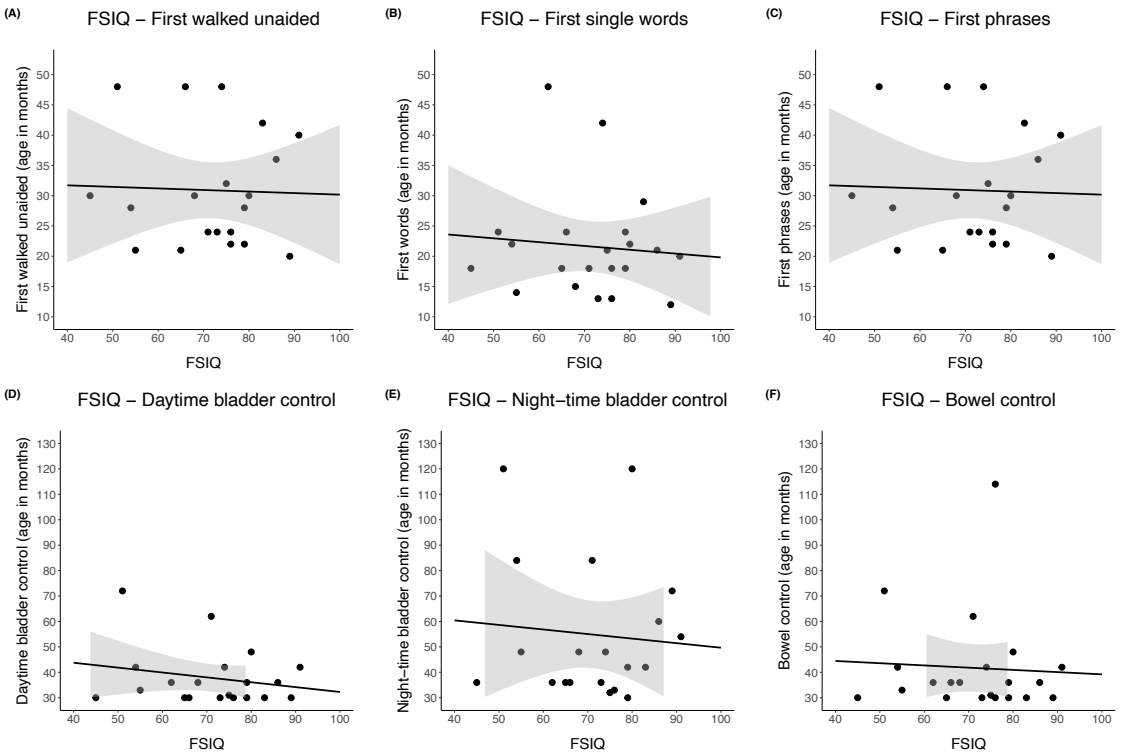
	Median	74
	Range	55 – 91
	Processing Speed Index (PSI)	
	N	22
	Average (*SD*)	74 (16)
	Median	78
	Range	49 – 103
WISC-V	Nonverbal Index (NVI)	
Ancillary Index Scale	*N*	23
	Average (*SD*)	73 (14)
	Median	75
	Range	50 – 94



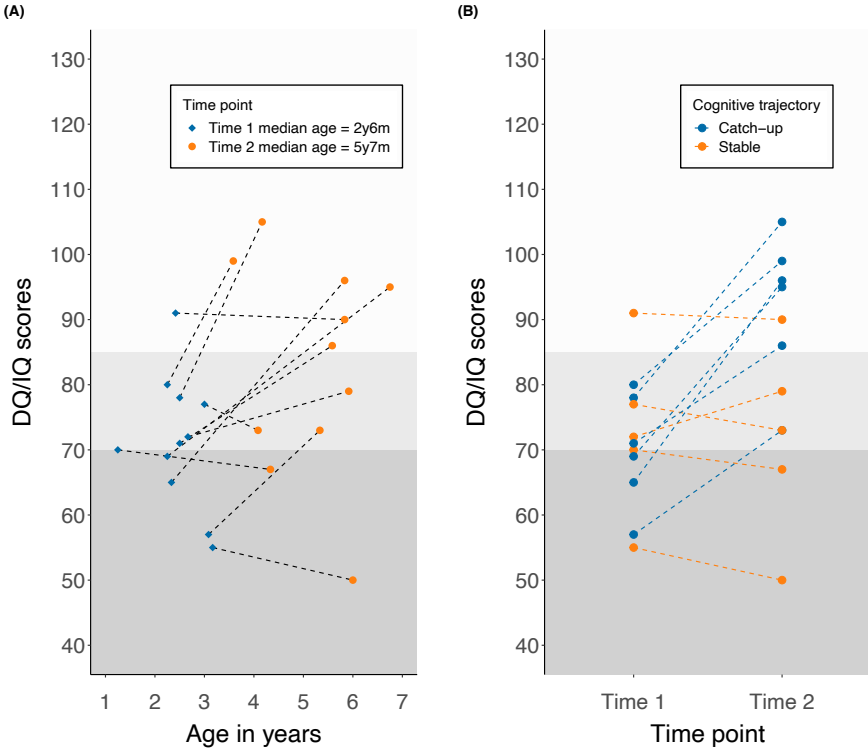
Supplementary Figure 6.1 – Boxplots FSIQ scores dependent on potential confounding factors: inheritance pattern, presence of a formal ASD or ADHD diagnosis and sex.



Supplementary Figure 6.2 – WISC-V Primary Index Scales across patients.
The individual lines represent the individual patient profiles across the five WISC-V Primary Index Scales. The grey zones delineate borderline IQ (70-84) and mild-moderate IQ (<70). Abbreviations: VCI, Verbal Comprehension Index; VSI, Visual Spatial Index; FRI, Fluid Reasoning Index; WMI, Working Memory Index; PSI, Processing Speed Index.



Supplementary Figure 6.3 – Scatterplots FSIQ and early developmental milestones. (A) Association FSIQ and First walked unaided (r = -0.271, p = 0.235); (B) Association FSIQ and First single words (r = -0.088, p = 0.703); (C) Association FSIQ and First phrases (at least two words) (r = -0.110, p = 0.634); (D) Association FSIQ and Daytime bladder control (r = -0.215, p = 0.362); (E) Association FSIQ and Night-time bladder control (r = -0.025, p = 0.913); (F) Association FSIQ and Bowel control (r = -0.055, p = 0.812).



Supplementary Figure 6.4 – Longitudinal cognitive trajectories in youngest comparison group of children with 16p11.2DS (*n* = 11).
(A) IQ scores as a function of age for each participant. The colour and shape refer to the time point. The dashed lines represent the individual cognitive trajectories. (B) IQ scores at two different time points (median age T1 2y6m, median age T2 5y7m). The colours refer to the cognitive trajectories: stable (|IQ T2 – T1| <10) and catch-up (IQ T2 – T1 > 10). In total, 6/11 (55%) children caught-up with peers, whereas 5/11 (45%) showed a relatively stable cognitive profile. Abbreviations: DQ, developmental quotient; IQ, intellectual quotient

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Chapter 7

Chapter 7 - Language profiles of school-aged children with 16p11.2 copy number variants in a clinically ascertained cohort

The content of this chapter is under revision for J*ournal of Speech, Language, and Hearing Research* as: Verbesselt, J., Breckpot, J., Zink, I., & Swillen, A. (2024). Language profiles of school-aged children with 16p11.2 copy number variants in a clinically ascertained cohort. Journal of Speech, Language, and Hearing Research, under revision. Supplementary material is provided at the end of this chapter.

Abstract

Background: Individuals with 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 (LD), intellectual disability (ID) and autism spectrum disorders (ASD). The purpose of the current study is to characterise language profiles of school-aged children with 16p11.2 CNVs, in relation to the normative sample and unaffected siblings of children with 16p11.2DS.

Methods: Standardised language tests were conducted in 33 school-aged children with 16p11.2 CNVs and 8 unaffected siblings of children with 16p11.2DS to evaluate language production and comprehension skills across various language domains. A standardised intelligence test was also administered, and parents completed a standardised questionnaire to assess autistic traits. Language profiles were compared across 16p11.2 CNVs and intrafamilial pairs. The influence of non-verbal intelligence and autistic traits on language outcomes was investigated.

Results: Although no significant differences were found between children with 16p11.2DS and those with 16p11.2Dup, both groups exhibited significantly poorer language skills compared to the normative sample and unaffected siblings of children with 16p11.2DS with large effect sizes. 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 significantly more pronounced than receptive deficits in children with 16p11.2DS with a large effect size (Cohen's $d = 0.818$). Non-verbal intelligence had a significant influence on language outcomes only in children with 16p11.2Dup.

Conclusion: The current study contributes to the deeper understanding of language profiles in 16p11.2 CNVs in a clinically ascertained cohort, indicating generalised 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.

7.1 Introduction

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 (LD), autism spectrum disorder (ASD) and intellectual disability (ID). 16p11.2DS primarily arises *de novo* (60-76%), while 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 behavioural symptoms, detailed data on language profiles in these CNVs remain limited (Chawner et al., 2021; Chung et al., 2021; 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., 2021, 2023).

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). Language disorders are frequently observed in 16p11.2DS, with prevalence rates ranging from 41% to 83% (Chung et al., 2021; Hanson et al., 2015; Mei et al., 2018). Mei et al. (2018) found average language scores of 2.1 standard deviations (*SD*) below the population mean in children with 16p11.2DS, while other studies reported a downward shift of 1.5-1.9 *SD* (Ahtam et al., 2019; Berman et al., 2015; Blackmon et al., 2018; Hanson et al., 2015; Matsuzaki et al., 2020). Mei et al. (2018) identified core language deficits, affecting morphological, syntactic and semantic language domains, in 83%

(33/40) of children with 16p11.2DS, with language scores nearly one *SD* below their non-verbal cognitive skills. Based on direct language instruments, syntactic difficulties were prevalent in 78% of individuals with 16p11.2DS (Kim et al., 2020). While 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-aged 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 (The Simons VIP Consortium, 2012). Children with 16p11.2Dup have language scores of 1.1-1.2 *SD* 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), while 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. While some research has examined correlations between language measures and brain structures or functions using neuroimaging studies, these efforts did not primarily focus on characterising 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.

7.2 Methodology

7.2.1 Participants

A cohort of 41 school-aged children was included in the current study (*M* = 10y11m, *SD* = 3y1m, 5y10m – 16y11m). This group consisted of 23 unrelated children with 16p11.2DS, 10 unrelated children with 16p11.2Dup and 8 full-biological unaffected siblings of the children with 16p11.2DS. Only individuals whose first language was Dutch or with at least three 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 a 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 7.1. 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.

Table 7.1 – Sociodemographic characteristics across 16p11.2 CNVs and siblings

	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 (yrs.mo)			
Average (*SD*)	10.1 (2.8)	11.2 (3.2)	11.1 (3.6)
Median	9.5	10.10	10.5
Range	6.3 – 13.8	5.10 – 16.11	6.9 – 15.9
Type of education (*n*, %)			
Special education	8 (80%)	19 (83%)	1 (12%)
Regular education	2 (20%)	1 (4%)	7 (88%)
Regular with assistance	0 (0%)	3 (13%)	0 (0%)
SES*			
High	3 (30%)	9 (39%)	4 (50%)
Middle	5 (50%)	12 (52%)	3 (38%)
Low	3 (30%)	2 (9%)	0 (0%)
Unknown	0 (0%)	0 (0%)	1 (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**	6/10 (60%)	8/23 (35%)	/

Note. Abbreviations: SES, socioeconomic status; NDD, neurodevelopmental disorders; ID, intellectual disability; ASD, autism spectrum disorder; ADHD, attention-deficit/hyperactivity disorder. *Educational attainment of the mother was used as a proxy for socioeconomic status (SES). The classification of SES was based on the International Standard Classification of Education (ISCED) of UNESCO (OECD, 2017; UNESCO Institute for Statistics., 2012), using three categories: low (primary education or lower grades of high school), middle (secondary/high school graduate), high (Bachelor,

Master's, or Doctoral Degree). **adopted (n = 1), foster care (n = 2), maternal inheritance ruled out, parents declined genetic testing …

7.2.2 Procedures and measurements

All individuals were included through the Centre for Human Genetics of University Hospitals Leuven or Maastricht University Medical Centre. Using a standardised protocol, prospective data were collected from all participants both in the clinic and at home. The research protocol involved an interview on developmental language milestones (first words, use of two-word sentences), two standardised language tests, an intelligence test and a standardised questionnaire on social responsiveness completed by parents or guardians.

Language assessment

Language skills of participants were assessed using the Dutch version 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 CELF- 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 language disorders, define therapy objectives, and track their development over time. Furthermore, the CELF-tests provide normative data for children aged 3 to 6 on the CELF-P2 and 5 to 18 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). Scaled scores falling within the range of 7 to 13 were categorised as average. Scaled scores of ≤6 indicated mild to moderate language difficulties, while SS of ≤3 indicated severe language deficits. The following receptive language subtests were administered: Word Classes-Receptive (WC-R), Sentence Structure (SST; 5.00 − 8.11 years) or Semantic Relationships (SR; ≥ 9.00 years), and Concepts and Following Directions (CFD; 5.00 – 12.11 years). The following expressive language subtests were administered: Word Classes- Expressive (WC-E), Formulated Sentences (FS), Recalling Sentences (RS), Word Structure (WS; 5.00 − 8.11 years), Expressive

Vocabulary (EV; 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 (CLS), receptive (RLI) and expressive language index scores (ELI) 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 versus 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 (WISC-V-NL; 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 (WNV-NL; Wechsler & Naglieri, 2008), Snijders–Oomen Nonverbal test Revised (SON-R; Tellegen & Laros, 2017) and Wechsler Preschool and Primary Scale of Intelligence – Fourth Edition (WPPSI-IV-NL; 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 to moderate impairments, while *T*-scores exceeding 75 (percentile <1.2) indicate severe deficits in social responsiveness.

7.2.3 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 $p < 0.05$, with Benjamini-Hochberg False Discovery Rates (FDR) applied to address potential type I-errors from multiple comparisons (Benjamini & Hochberg, 1995; Benjamini & Yekutieli, 2001). Adjusted *p*-values fell within the range of 0.0071 to 0.05. 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 analysed using either Student's or Wilcoxon signed-rank 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: 1) statistical tests on a group level, 2) analysis of percentage differences within and across subgroups, and 3) 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 sample 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 versus RLI, WCS versus RLI, and CLS versus NVI) using paired samples Student's or Wilcoxon signed-rank *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 cut-off 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. For CELF subtests, children were identified as experiencing language difficulties if their scores deviated by more than one *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 core language scores (CLS), 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 ADHD, and SES were explored through descriptive statistics and/or independent *t*-tests. Venn diagrams were plotted to visualise the number of children with severely affected (<70) 1) RLI, ELI and/or NVI, and 2) CLS, SRS, and/or NVI.

7.3 Results

7.3.1 Language and IQ in 16p11.2 CNVs and siblings compared to the norm group

Figure 7.1 depicts the boxplots of the seven summary scores for the three groups of children, with the dashed line referring to the normal population mean and the grey zones demonstrating the cut-offs for mild-moderate to severe cognitive or language difficulties. 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 summarised in Table 7.2.

The density plots for the CELF core language scores (CLS) are displayed in Figure 7.2. 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 *SD* (≈25 CLS points) and 2.36 *SD* (≈26 CLS points) respectively, whereas the distribution of unaffected siblings of children with 16p11.2DS show 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 ($p < 0.001$) and 16p11.2Dup ($0.001 > p > 0.010$) for all composite

scores with large effect sizes (d < -1.036, r < -0.993) (Supplementary Table 7.1). The scores of the siblings of children with 16p11.2DS did not significantly differ from the norm group scores (0.081 < p < 0.200).

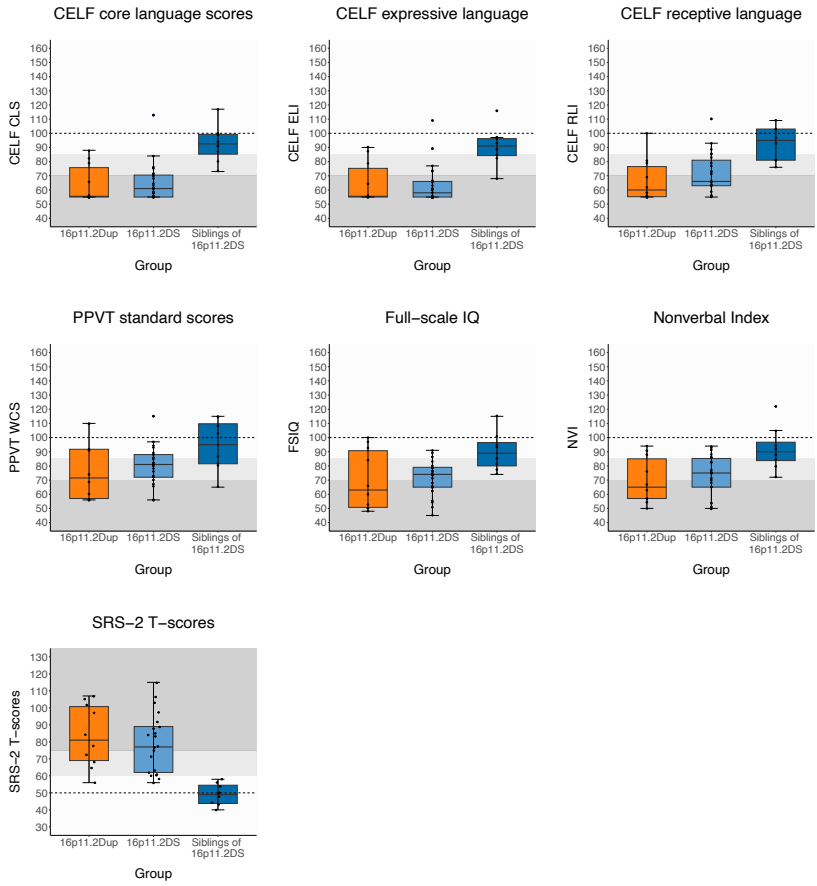


Figure 7.1 – Boxplots for CELF, PPVT and IQ scores across 16p11.2 CNVs and siblings of 16p11.2DS.

The dashed lines illustrate the norm group averages. The grey zones refer to the severity of the deficits; the darker the grey, the more severe the deficits: light grey zone = mild-moderate, and darker grey zone = severe, based on clinical cut-off scores for CELF, PPVT and Wechsler scales. Abbreviations: CELF-4-NL or CELF-P2-NL, Clinical Evaluation of Language Fundamentals; CLS, Core Language Scores; ELI, Expressive Language Index; RLI, Receptive Language Index; PPVT-III-NL, Peabody Picture Vocabulary Test; WCS, Word Comprehension Score; FSIQ, Full-Scale IQ; NVI, Nonverbal Index. Average = 100, *SD* = 15 in the normative sample, cut-off: <85 = mild-moderate; <70 = severe. SRS-2, Social Responsiveness Scale. Average = 50, *SD* = 10 in the normative sample, cut-off > 60 = mild-moderate; >75 = severe.

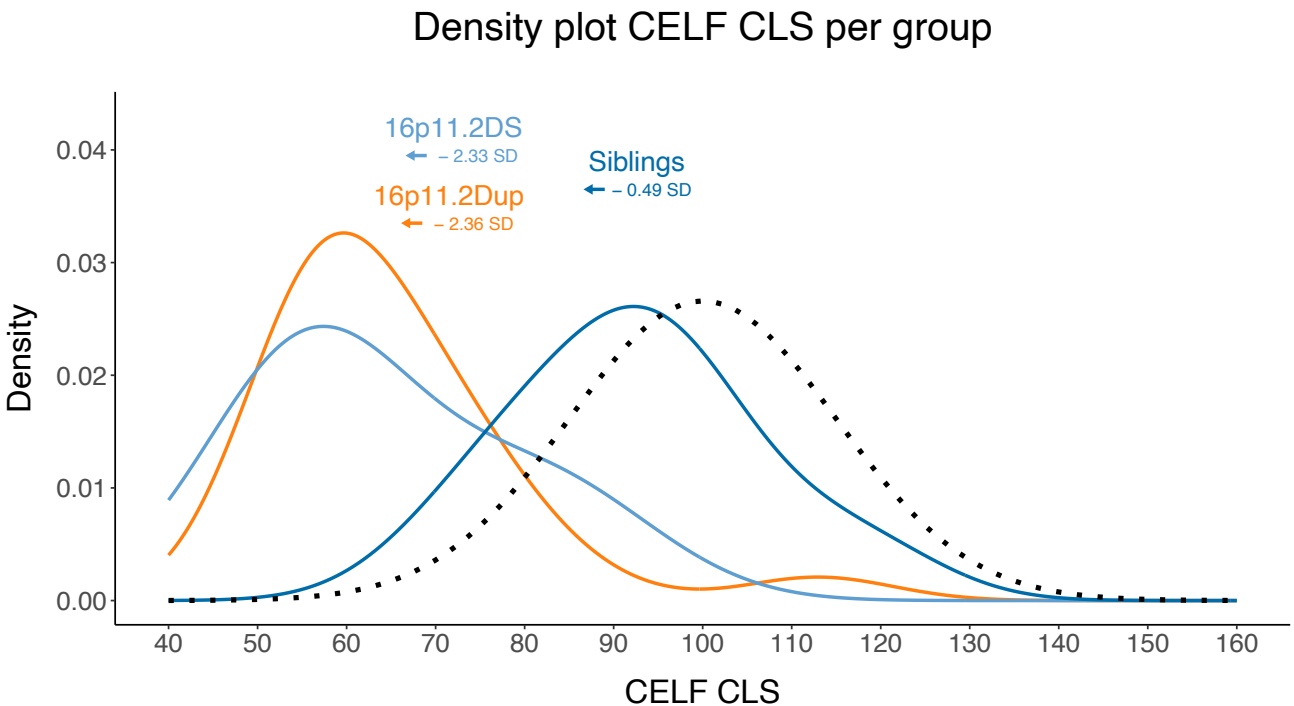


Figure 7.2 – Normative distributions of CELF CLS for the three groups of children (16p11.2DS, 16p11.2Dup and siblings of 16p11.2DS). Abbreviations: CLS, core language scores; *SD*, standard deviation. The dashed line depicts the standard distribution of the normative sample (mean = 100, *SD* = 15). *SD* changes are determined relative to typically developing peers in the general population, represented by the norm group.

7.3.2 Cross-CNV and intrafamilial comparisons at group level: mean differences

In both CNV groups, average CELF core language scores (CLS) fell within the severe range (<70), whereas PPVT WCS scores 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, while 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 ($p > 0.209$) (Table 7.2). However, children with 16p11.2DS demonstrated statistically significantly lower composite scores than their unaffected siblings ($0.001 < p < 0.047$) with large effect sizes.

Children with 16p11.2DS demonstrated significantly lower expressive index scores (ELI mean = 63.61) compared to receptive language index scores (RLI mean = 71.91; $p < 0.001$, $d = 0.818$, see Supplementary Table 7.2), with a large effect size. Conversely, their siblings exhibited comparable RLI and ELI scores (ELI mean = 90.75, RLI mean = 92.88; $p = 0.469$, $d = 0.271$) as did children with 16p11.2Dup (ELI mean = 65.10, RLI mean = 67.00; $p = 0.218$, $d = 0.419$). Receptive vocabulary (PPVT WCS) was statistically significantly higher than CELF RLI for both CNV groups with large effect sizes (DS: $p = 0.001$, $d = -0.822$; Dup: $p = 0.018$, $r = -0.911$), but not for siblings of children with 16p11.2DS ($p = 0.755$, $d = -0.115$). Similarly, CELF CLS were statistically significantly lower than NVI in 16p11.2Dup ($p = 0.016$, $d = -0.940$) with a large effect size, but not in those with 16p11.2DS ($p = 0.032$, $d = -0.491$) or their siblings ($p = 0.920$, $d = 0.037$). While 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 (Supplementary Figure 7.1/Table 7.3). In the 16p11.2DS cohort, individuals who experienced delayed speech-language milestones during infancy ($n = 21$) exhibited average CLS of 62.42, whereas those without speech-language delays ($n = 2$) had average CLS of 92.50.

Table 7.2 – Cross-CNV and intrafamilial comparisons across CELF composite, PPVT and IQ scores.

	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 Mean (*SD*)	65.04 (13.40)	64.00 (13.31)	*W* = 126.000	63.13 (7.95)	92.63 (13.47)	*t* = -6.123
Median	61.00	55.50	*p* = 0.673	63.00	92.50	*p* < 0.001**
Range	55.00 – 113.00	55.00 – 88.00	*r* = 0.096	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 Mean (*SD*)	71.91 (14.09)	67.00 (15.25)	*W* = 147.500	71.88 (12.63)	92.88 (12.24)	*t* = -3.650
Median	66.00	60.00	*p* = 0.209	72.00	95.00	*p* = 0.008**
Range	55.00 – 110.00	55.00 – 100.00	*r* = 0.283	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 Mean (*SD*)	63.61 (13.40)	65.10 (14.48)	*W* = 114.40	61.13 (6.90)	90.75 (13.83)	*t* = -6.922
Median	58.00	55.50	*p* = 1.000	59.50	91.00	*p* < *0.001***
Range	55.00 – 109.00	55.00 – 90.00	*r* = -0.004	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 Mean (*SD*)	80.29 (13.86)	77.40 (21.81)	*t* = 0.449	77.88 (14.34)	94.38 (18.47)	*t* = -2.400
Median	81.00	71.50	*p* = 0.657	76.00	95.00	*p* = 0.047**
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 Mean (*SD*)***	71.33 (12.55)	70.10 (21.20)	*W* = 113.50	69.43 (9.05)	90.13 (13.69)	*t* = -4.228

Median	74.00	63.00	$p = 0.735$	74.00	89.00	$p = 0.006$**
Range	45.00 – 91.00	48.00 – 100.00	$r = 0.081$	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 Mean (*SD*)	73.00 (14.35)	69.70 (16.43)	$t = 0.569$	70.38 (14.42)	92.25 (15.50)	$t = -4.868$
Median	75.00	65.00	$p = 0.574$	73.50	90.00	$p = 0.002$**
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-2 Mean (*SD*)	79.14 (17.32)	83.40 (18.38)	$t = -0.628$	84.13 (15.82)	49.13 (6.53)	$t = 5.457$
Median	77.00	81.00	$p = 0.535$	83.50	49.00	$p < 0.001$**
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. Statistical outcomes: *p*-value; *significant at $p < 0.05$, **significant with FDR; *t*-value or *W*-value; Cohen's d or rank biserial correlation r as effect size. *** One participant's verbal skills were insufficient, resulting in only NVI scores being available for analysis. Abbreviations: 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, cut-off: <85: mild–moderate, <70: severe); SRS-2, Social Responsiveness Scale (norm group average = 50, cut-off: >60: mild-moderate, >75: severe).

7.3.3 Cross-CNV and intrafamilial comparisons at subgroup level: proportion differences

Table 7.3 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 ($p > 0.358$). However, statistically significantly more deficits were observed in children with 16p11.2DS compared to their unaffected siblings ($0.001 < p < 0.007$), except for receptive language skills (CELF RLI: $p = 0.315$ and PPVT WCS: $p = 0.619$) and FSIQ ($p = 0.026$) (Supplementary table 7.4).

Clinically relevant poorer expressive than receptive language skills, defined as a difference of at least 15 points between both summary scores, were found in 6/23 (26%) of 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 5/21 (21%) of children with 16p11.2DS, 2/10 (20%) of children with 16p11.2Dup and 1/8 (13%) of siblings. The nonverbal index (NVI) was significantly higher than the overall language score (CLS) in 9/22 (41%) of children with 16p11.2DS, none of those with 16p11.2Dup and none of the siblings.

Using appropriate cut-off 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. Most common difficulties in children with 16p11.2DS were problems with Word Definitions in 92%, Recalling Sentences in 91% and Word Classes- Expressive in 82%. In the 16p11.2Dup group, most deficits were impairments in Word Definitions and Recalling Sentences in all participants, and Sentence Structure/Semantic Relations 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.

Table 7.3 – Proportions of children with difficulties across composite and subtest scores on PPVT and CELF.

		16p11.2Dup	**16p11.2DS**	**Siblings of 16p11.2DS**
Sample Size (*n*)		10	23	8
Composite scores	PPVT WCS (<-1 *SD* & <-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* & <-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* & <-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* & <-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%)
Subtest scores receptive	CFD (5.00 – 12.11 years)	5/8* (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%)
Subtest scores expressive	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 (≥ 10.00 years)	4/4 (100%)	11/12 (92%)	1/4 (25%)
Subtest scores combined receptive and expressive	WC-T	7/10 (70%)	16/22 (73%)	1/8 (12%)

Note. * Available data vary by subtest due to different age ranges of specific subtests or missing data. Abbreviations : WCS, Word Comprehension Score; CLS, Core Language Score; RLI, Receptive Language Index; ELI, Expressive Language Index (cut-off: <85: mild–moderate, <70: severe); CFD, Concepts and Following Directions; RS, Recalling Sentences; FS, Formulated Sentences; WS, Word Structure; SST, Sentence Structure (5.00−8.11 years); SR, Semantic Relations (≥ 9.00 years); WC-R, Word Classes-Receptive; WC-E, Word Classes- Expressive; WC-T, Word Classes-Total; EV, Expressive Vocabulary; WD, Word Definitions (cut-off: <7: mild– moderate problems; <4: severe problems).

7.3.4 Impact of confounding factors on language outcomes in 16p11.2 CNVs

While linear regression models did not reveal a significant influence of NVI or SRS on CLS in 16p11.2DS ($p = 0.524$), a significant impact of NVI was found in 16p11.2Dup ($p < 0.001$) with a large coefficient of determination (R^2) of 92.3% (Supplementary Table 7.5). 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* lower than expected based on norm group scores ($p = 0.003$), while children with 16p11.2Dup would exhibit language scores of 0.7 *SD* lower than expected ($p = 0.011$). Independent *t*- tests did not reveal any significant differences between children with 16p11.2 CNVs with or without ASD ($p > 0.410$). Similarly, no differences were found between children with 16p11.2 CNVs with or without ADHD ($p > 0.187$) or between male and female children ($p > 0.114$). Descriptive statistics for these confounding factors and SES can be found in Supplementary Table 7.6.

Scatterplots of CELF CLS in function of NVI and SRS are displayed in figure 7.3A-B. Figure 7.3C illustrates the comorbidity between expressive (ELI), receptive (RLI) and nonverbal skills (NVI), based on clinical cut-offs for 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 7.3D shows the comorbidity between language (CELF CLS), nonverbal skills (NVI) and social responsiveness (SRS) based on the clinical cut-offs 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.

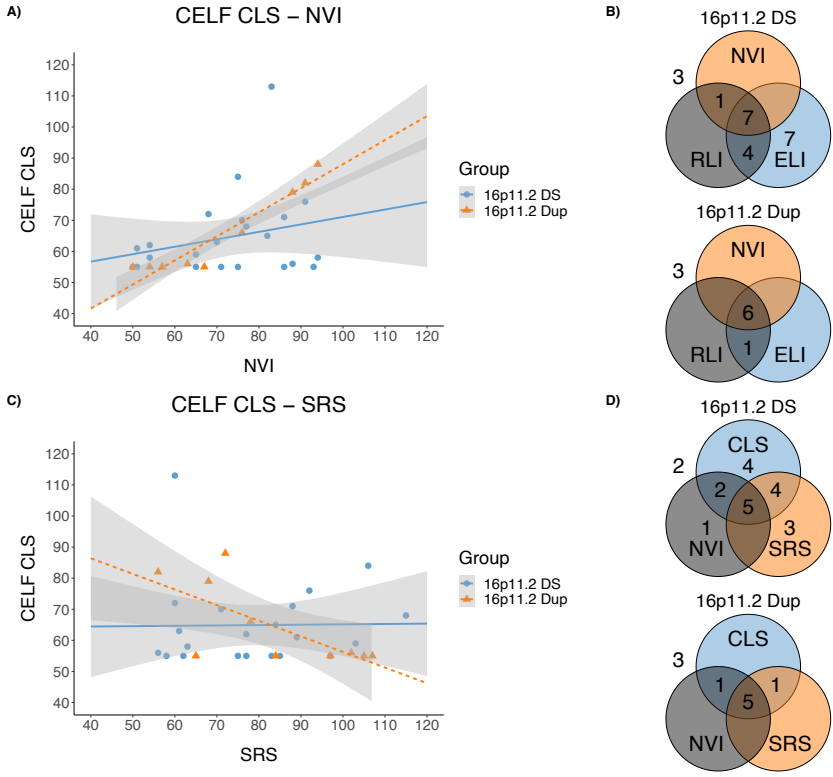


Figure 7.3 – Correlations and comorbidity across 16p11.2 CNVs.
A) Scatterplot association between CELF CLS and NVI; B) Scatterplot association between CELF CLS and SRS-2 total *T*-score; C) Comorbidity in 16p11.2 CNVs – participants with severe scores on NVI, RLI and ELI. D) Comorbidity in 16p11.2 CNVs – participants with severe scores on CLS, NVI and SRS. Abbreviations: CLS, Core Language Score; RLI, Receptive Language Index; ELI, Expressive Language Index; NVI, Nonverbal Index (cut-off: <70: severe); SRS, Social responsiveness scale (cut-off >75: severe). The number outside the Venn diagram refers to the remaining participants for whom all three test scores were obtained.

7.4 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 nonverbal index and social responsiveness on language outcome. Standardised language and intelligence tests were administered in 23 children with 16p11.2DS, 10 children with 16p11.2Dup and 8 unaffected siblings of children with 16p11.2DS, while their parents completed a standardised questionnaire about autistic traits.

7.4.1 16p11.2 CNVs and siblings in comparison to the norm group

The first specific aim was to evaluate whether children with 16p11.2 CNVs and siblings of children with 16p11.2DS showed comparable language scores in relation to norm group scores. Boxplots revealed heterogeneous profiles, especially in the 16p11.2Dup group, aligning with prior studies that have shown broader phenotypic variability in 16p11.2Dup 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* in both CNV groups, which is more pronounced than reported in previous studies with shifts of -1.5-2.1 *SD* in 16p11.2DS and of -1.1-1.2 *SD* 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* 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 and to inclusion restricted to index patients, reflecting more participants at the severe end of the spectrum.

On the contrary, the language and cognitive skills of unaffected siblings of children with 16p11.2DS did not show 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).

7.4.2 Language and IQ differences across 16p11.2DS and 16p11.2Dup

The second aim was to examine language and IQ differences between children with 16p11.2DS and those with 16p11.2Dup. On average, both CNV groups demonstrated severe core language deficits, 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, while 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, reporting average differences of 0 to 6 IQ points (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 with average differences of 10 to 11 IQ points (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. for 16p11.2DS (33/40, 83%; 2018). The majority of both groups (7/10 & 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 in 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 core language scores, 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.

7.4.3 Language and IQ differences between 16p11.2DS and unaffected siblings

The third aim was to investigate differences between children with 16p11.2DS and their 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. (2015) 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 variable results in siblings underscore the importance of the interplay between genetic factors and environmental influences, such as the shared familial background, on neurodevelopmental outcomes.

7.4.4 Differences across composite scores

For the fourth aim, we investigated potential differences within individuals across several 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 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. While 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, and 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. word definitions ≥ 10.00 years, only administered in 4 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 and morpho-syntactic 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 Harbour 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 core language score 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. 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. In addition, it affects our ability to detect profile discrepancies in the lowest range.

7.4.5 Influence of confounding factors on language outcomes

Finally, we aimed to explore the potential influence of confounding factors, such as autistic traits and nonverbal cognitive skills, on language outcomes in individuals with 16p11.2 CNVs. 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 while 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. (2020). 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. (2020), 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.

7.4.6 Strengths, limitations and future

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 minimised 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 behavioural skills in 16p11.2DS. The use of standardised 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, multi-site 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- aged 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

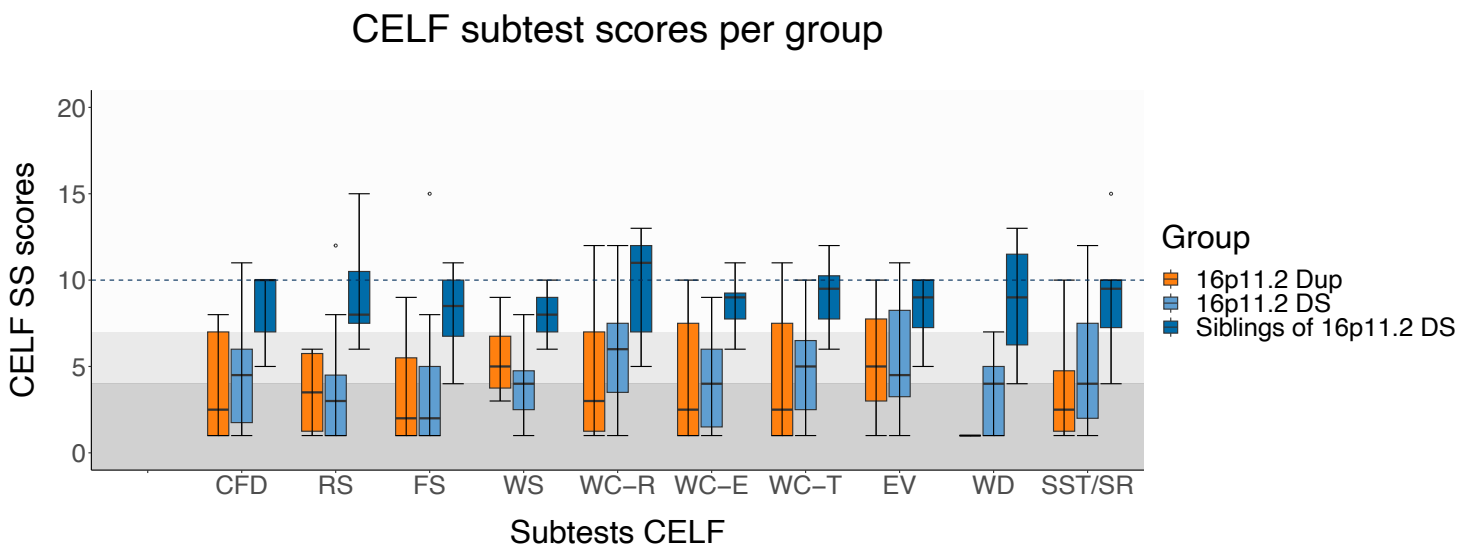
focused on lexico-semantic and morpho-syntactic language abilities, future research should also characterise pragmatic language skills and speech in both CNV groups. Furthermore, longitudinal studies are warranted to capture language skills over time, as language and cognitive profiles in CNVs may evolve 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.

7.5 Conclusion

The current study characterised language profiles of school-aged 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 and Herrera (2023), 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 centres or private practitioners. Since there is variability present in language performances, tailored and individualised interventions are needed to enhance language abilities and mitigate potential long-term impacts of language and communication difficulties.

7.6 Supplementary material



Supplementary Figure 7.1 – CELF subtest scores per group.
Abbreviations: CFD, Concepts and Following Directions; RS, Recalling Sentences; FS, Formulated Sentences; WS, Word Structure; SST, Sentence Structure (5.00 – 8.11 years); SR, Semantic Relations (≥9.00 years); WC-R, Word Classes-Receptive; WC-E, Word Classes-Expressive; WC-T, Word Classes-Total; EV, Expressive Vocabulary; WD, Word Definitions (cut-off: <7: mild–moderate problems; <4: severe problems).

Supplementary Table 7.1 – CNV groups and siblings of 16p11.2DS compared to the normative sample

	16p11.2DS *N* *M (SD)* Median	**Statistical outcomes group level** One sample *t*-test (*t*/*V* = , p = , *d*/*r* =)	**16p11.2Dup** *N* *M (SD)* Median	**Statistical outcomes group level** One sample *t*-test (*t*/*V* = , *p* = , *d*/*r* =)	**Siblings of 16p11.2DS** *N* *M (SD)* Median	**Statistical outcomes group level** One sample *t*-test (*t*/*V* = , *p* = , *d*/*r* =)
CELF CLS:						
N	23	*V* = 1.000	10	*V* = 0.000	8	*t* = -1.549
M (SD)	65.04 (13.40)	*p* < 0.001*	64.00 (13.31)	*p* = 0.005*	92.63 (13.47)	*p* = 0.165
Median	61.00	*r* = -0.993	55.50	*r* = -1.000	92.50	*d* = -0.643
CELF RLI:						
N	23	*t* = -9.562	10	*t* = -6.845	8	*t* = -1.646
M (SD)	71.91 (14.09)	*p* < 0.001*	67.00 (15.25)	*p* < 0.001*	92.88 (12.24)	*p* = 0.144
Median	66.00	*d* = -1.994	60.00	*d* = -2.660	95.00	*d* = -0.500
CELF ELI:						
N	23	*V* = 1.000	10	*V* = 0.000	8	*t* = -1.891
M (SD)	63.61 (13.40)	*p* < 0.001*	65.10 (14.48)	*p* = 0.005*	90.75 (13.83)	*p* = 0.100
Median	58.00	*r* = -0.993	55.50	*r* = -1.000	91.00	*d* = -0.667
PPVT WCS:						
N	21	*t* = -6.518	10	*t* = -3.276	8	*t* = -0.861
M (SD)	80.29 (13.86)	*p* < 0.001*	77.40 (21.81)	*p* = 0.010*	94.38 (18.47)	*p* = 0.418

	Median	81.00	d = -1.422	71.50	d = -1.036	95.00	d = -0.305
FSIQ:							
	N	21	t = -10.466	10	t = -4.461	8	t = -2.041
	M (SD)	71.33 (12.55)	p < 0.001*	70.10 (21.20)	p = 0.002*	90.13 (13.69)	p = 0.081
	Median	74.00	d = -2.284	63.00	d = -1.411	89.00	d = -0.694
NVI:							
	N	22	t = -8.837	10	t = -5.834	8	t = -1.414
	M (SD)	73.00 (14.35)	p < 0.001*	69.70 (16.43)	p < 0.001*	92.25 (15.50)	p = 0.200
	Median	75.00	d = -1.884	65.00	d = -1.845	90.00	d = -0.556
SRS-2:							
	N	21	t = 7.710	10	t = 5.746	8	t = -0.379
	M (SD)	79.14 (17.32)	p < 0.001*	83.40 (18.38)	p < 0.001*	49.13 (6.53)	p = 0.716
	Median	77.00	d = 1.682	81.00	d = 1.817	49.00	d = -0.134

Note. Statistical outcomes: *p*-value; *significant with FDR, *t*-value or V-value. Cohen's d or rank biserial correlation r as effect size. Abbreviations: 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, cut-off: <85: mild–moderate, <70: severe). SRS-2, Social Responsiveness Scale (norm group average = 50, cut-off: >60: mild-moderate, >75: severe).

Supplementary Table 7.2 – Intraindividual comparisons for each group across receptive vs expressive, receptive vocabulary vs receptive language, and core language vs nonverbal index scores

	16p11.2DS (*n* = 23)	**Statistical outcomes paired *t*-test**	**16p11.2Dup (*n* = 10)**	**Statistical outcomes paired *t*-test**	**Siblings of 16p11.2DS (*n* = 8)**	**Statistical outcomes paired *t*-test**
CELF RLI Mean (*SD*)	71.91 (14.09)	$t = 3.925$	67.00 (15.25)	$t = 1.326$	92.88 (12.24)	$t = 0.766$
Median ↕	66.00	$p < 0.001$**	60.00	$p = 0.218$	95.00	$p = 0.469$
CELF ELI Mean (*SD*)	63.61 (13.40)	$d = 0.818$	65.10 (14.48)	$d = 0.419$	90.75 (13.83)	$d = 0.271$
Median	58.00		55.50		91.00	
CELF RLI Mean (*SD*)	71.91 (14.09)	$t = -3.765$	67.00 (15.25)	$W = 2.000$	92.88 (12.24)	$t = -0.325$
Median ↕	66.00	$p = 0.001$**	60.00	$p = 0.018$**	95.00	$p = 0.755$
PPVT WCS Mean (*SD*)	80.29 (13.86)	$d = -0.822$	77.40 (21.81)	$r = -0.911$	94.38 (18.47)	$d = -0.115$
Median	81.00		71.50		95.00	
CELF CLS Mean (*SD*)	65.04 (13.40)	$t = -2.302$	64.00 (13.31)	$t = -2.973$	92.63 (13.47)	$t = 0.104$
Median ↕	61.00	$p = 0.032$*	55.50	$p = 0.016$**	92.50	$p = 0.920$
NVI Mean (*SD*)	73.00 (14.35)	$d = -0.491$	69.70 (16.43)	$d = -0.940$	92.25 (15.50)	$d = 0.037$
Median	75.00		65.00		90.00	

Note. Statistical outcomes: *p*-value; *significant at $p < 0.05$, **significant with FDR

Supplementary Table 7.3 – Subtest scores across children with 16p11.2 CNVs and siblings

			16p11.2Dup	**16p11.2DS**	**Siblings of 16p11.2DS**
Subtest scores receptive	CFD ($5.00 - 12.11\ years$)				
		N	8*	14	5
		M (SD)	3.75 (3.15)	4.64 (3.08)	8.40 (2.30)
		Median	1.00	4.50	10.00
		Range	1.00 – 8.00	1.00 – 11.00	5.00 – 10.00
	SST / SR				
		N	10	23	8
		M (SD)	3.50 (2.88)	4.83 (3.21)	8.88 (3.40)
		Median	2.50	4.00	9.50
		Range	1.00 – 10.00	1.00 – 12.00	4.00 – 15.00
	SST				
		N	4	7	2
		M (SD)	5.50 (3.42)	3.43 (3.10)	7.00 (4.24)
		Median	5.00	2.00	7.00
		Range	2.00 – 10.00	1.00 – 9.00	4.00 – 10.00
	SR				
		N	6	16	6
		M (SD)	2.17 (1.60)	5.44 (3.16)	9.50 (3.27)
		Median	1.50	5.00	9.50

		Range	1.00 – 5.00	1.00 – 12.00	5.00 – 15.00
	WC-R				
		N	10	23	8
		M (SD)	4.60 (3.92)	5.39 (3.06)	9.75 (2.96)
		Median	3.00	6.00	11.00
		Range	1.00 – 12.00	1.00 – 12.00	5.00 – 13.00
Subtest scores expressive	RS				
		N	10	23	8
		M (SD)	3.50 (2.27)	3.30 (2.72)	9.13 (3.09)
		Median	3.50	2.50	8.00
		Range	1.00 – 6.00	1.00 – 8.00	6.00 – 15.00
	FS				
		N	10	21	8
		M (SD)	3.50 (3.14)	3.43 (3.56)	8.13 (2.36)
		Median	2.00	2.00	8.50
		Range	1.00 – 9.00	1.00 – 15.00	4.00 – 11.00
	WC-E				
		N	10	23	8
		M (SD)	4.20 (3.83)	4.13 (2.74)	8.63 (1.60)
		Median	2.50	4.00	9.00
		Range	1.00 – 10.00	1.00 – 9.00	6.00 – 11.00
	WS (5.00 – 8.11 years)				
		N	4	8	2

	M (SD)	5.50 (2.65)	4.00 (2.51)	8.00 (2.83)
	Median	5.00	4.00	8.00
	Range	3.00 – 9.00	1.00 – 8.00	6.00 – 10.00
	EV (5.00 – 9.11 years)			
	N	6	10	4
	M (SD)	5.33 (3.50)	5.50 (3.44)	8.25 (2.36)
	Median	5.00	4.50	9.00
	Range	1.00 – 10.00	1.00 – 11.00	5.00 – 10.00
	WD (≥10.00 years)			
	N	4	13	4
	M (SD)	1.00 (0.00)	3.31 (2.21)	8.75 (4.03)
	Median	1.00	4.00	9.00
	Range	1.00 – 1.00	1.00 – 7.00	4.00 – 13.00
Subtest scores combined receptive and expressive	WC-T			
	N	10	23	8
	M (SD)	4.40 (4.01)	4.57 (2.87)	8.13 (2.03)
	Median	2.50	5.00	9.50
	Range	1.00 – 11.00	1.00 – 10.00	6.00 – 12.00

Note. * Available data vary by subtest due to different age ranges of specific subtests or missing data. Abbreviations: CFD, Concepts and Following Directions; RS, Recalling Sentences; FS, Formulated Sentences; WS, Word Structure; SST, Sentence Structure (5.00 – 8.11 years); SR, Semantic Relations (≥9.00 years); WC-R, Word Classes-Receptive; WC-E, Word Classes-Expressive; WC-T, Word Classes-Total; EV, Expressive Vocabulary; WD, Word Definitions (cut-off: <7: mild–moderate problems; <4: severe problems).

Supplementary Table 7.4 – Cross-CNV and intrafamilial proportion differences across composite scores

	16p11.2DS (*n* = 23) % with problems (<-1 *SD*)	**16p11.2Dup (*n* = 10)** % with problems (<-1 *SD*)	**Statistical outcomes subgroup level** Fisher's exact (*p* = , *OR* =)
CELF CLS	22/23 (96%)	9/10 (90%)	*p* = 0.521; *OR* = -0.863
CELF RLI	18/23 (78%)	9/10 (90%)	*p* = 0.640; *OR* = 0.892
CELF ELI	21/23 (91%)	8/10 (80%)	*p* = 0.567; *OR* = -0.932
PPVT WCS	13/21 (62%)	6/10 (60%)	*p* = 1.000; *OR* = -0.077
FSIQ	18/21 (86%)	7/10 (70%)	*p* = 0.358; *OR* = -0.911
NVI	21/22 (95%)	9/10 (90%)	*p* = 0.534; *OR* = -0.818
SRS-2	17/21 (81%)	9/10 (90%)	*p* = 1.000; *OR* = 0.729
	16p11.2DS (*n* = 8) % with problems (<-1 *SD*)	**Siblings of 16p11.2DS** (*n* = 8) % with problems (<-1 *SD*)	**Statistical outcomes subgroup level** Fisher's exact (*p* = , *OR* =)
CELF CLS	8/8 (100%)	2/8 (25%)	*p* = 0.007**; *OR* = -∞
CELF RLI	6/8 (75%)	3/8 (38%)	*p* = 0.315; *OR* = -1.498
CELF ELI	8/8 (100%)	2/8 (25%)	*p* = 0.007**; *OR* = -∞
PPVT WCS	5/8 (62%)	3/8 (38%)	*p* = 0.619; *OR* = -0.955
FSIQ	7/7 (100%)	3/8 (38%)	*p* = 0.026*; *OR* = -∞
NVI	8/8 (100%)	2/8 (25%)	*p* = 0.007**; *OR* = -∞
SRS-2	8/8 (100%)	0/8 (0%)	*p < 0.001***; *OR* = -∞

Note. Statistical outcomes: *p*-value; *significant at $p < 0.05$, **significant with FDR. Odds ratio as effect size. Abbreviations: 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, cut-off: <85: mild–moderate, <70: severe). SRS-2, Social Responsiveness Scale (norm group average = 50, cut-off: >60: mild-moderate, >75: severe).

Supplementary Table 7.5 – Regression analyses predicting language scores while controlling for SRS and NVI scores in 16p11.2 CNVs

		Unst. coefficients	*SE*	*t*	*p*	R^2
16p11.2DS (*n* = 23) CELF CLS *z*-score	H_1 Intercept	-1.845	0.544	-3.390	0.003**	0.099
	SRS-2 total *z*-score	-0.032	0.120	-0.266	0.793	
	NVI *z*-score	0.314	0.224	1.401	0.178	
16p11.2Dup (*n* = 10) CELF CLS *z*-score	H_1 Intercept	-0.721	0.208	-3.461	0.011**	0.923
	SRS-2 total *z*-score	0.066	0.066	0.990	0.355	
	NVI *z*-score	0.703	0.111	6.323	<0.001**	

Note. Statistical outcomes: Unstandardised coefficients; *p*-value; *significant at $p < 0.05$, **significant with FDR. R^2 coefficient of determination. Odds ratio as effect size. Abbreviations: CLS, Core Language Score; SRS, Social Responsiveness Scale; NVI, Nonverbal Index (standardised norm group average = 0, *SD* = 1, cut-off: <-1: mild–moderate, <-2: severe).

Supplementary Table 7.6 – Descriptive statistics CELF CLS across subgroups based on potential confounding factors in 16p11.2 CNVs.

			16p11.2DS	**16p11.2Dup**
Inheritance pattern	*De novo*			
		N	12	2
		Average (*SD*)	67.25 (17.43)	68.50 (19.09)
		Median	60.50	68.50
		Range	55.00 – 113.00	55.00 – 82.00
	Inherited			
		N	3	2
		Average (*SD*)	58.67 (4.04)	72.00 (22.63)
		Median	58.00	72.00
		Range	55.00 – 63.00	56.00 – 88.00
ASD diagnosis	ASD			
		N	10	7
		Average (*SD*)	63.40 (8.90)	63.71 (14.65)
		Median	60.50	55.00
		Range	55.00 – 84.00	55.00 – 88.00
	No ASD			
		N	12	3
		Average (*SD*)	65.58 (16.81)	66.67 (12.01)
		Median	58.50	66.00
		Range	55.00 – 113.00	55.00 – 79.00
ADHD diagnosis	ADHD			

		N	7	6
		Average (*SD*)	62.00 (8.62)	62.50 (13.22)
		Median	58.00	55.50
		Range	55.00 – 76.00	55.00 – 88.00
	No ADHD			
		N	15	4
		Average (*SD*)	65.80 (14.42)	67.75 (14.77)
		Median	61.00	67.00
		Range	55.00 – 113.00	55.00 – 82.00
Sex	Female			
		N	14	5
		Average (*SD*)	66.57 (16.33)	62.80 (11.69)
		Median	60.00	56.00
		Range	55.00 – 113.00	55.00 – 82.00
	Male			
		N	9	5
		Average (*SD*)	62.67 (7.07)	66.40 (15.93)
		Median	62.00	55.00
		Range	55.00 – 72.00	55.00 – 88.00
SES	High			
		N	9	2
		Average (*SD*)	65.89 (10.61)	68.50 (19.09)
		Median	61.00	68.50
		Range	55.00 – 84.00	55.00 – 82.00
	Middle			
		N	12	5
		Average (*SD*)	65.17 (16.48)	66.40 (15.93)
		Median	59.00	55.00
		Range	55.00 – 113.00	55.00 – 88.00
	Low			
		N	2	3
		Average (*SD*)	60.50 (3.54)	59.00 (6.08)
		Median	60.50	56.00
		Range	58.00 – 63.00	55.00 – 66.00

Note. Abbreviations: ASD, autism spectrum disorder; ADHD, attention-deficit/hyperactivity disorder, SES, socioeconomic status.

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Chapter 8

Chapter 8 - General discussion

The purpose of the current dissertation was to further contribute to the characterisation of language, cognitive and behavioural profiles in a clinically ascertained cohort of school-aged children with 22q11.2DS, 22q11.2Dup, 16p11.2DS and 16p11.2Dup, through deep phenotyping. Until now, structured, protocol-driven studies on these developmental domains remained scarce and fragmented, lacking clear differentiation between language and speech deficits, particularly in 22q11.2Dup and 16p11.2 CNVs. Furthermore, the relationship between speech-language difficulties and concurrent neurodevelopmental and behavioural problems within these NDD-CNVs has not been studied before. The in-depth characterisation of language, cognition and behaviour is crucial for understanding the nature, occurrence and severity of neurodevelopmental difficulties associated with these four CNVs. This understanding is needed to inform healthcare professionals, and guide neurodevelopmental follow-up and intervention strategies aimed at mitigating the potential long-term impact of these difficulties. Six studies (Chapters 2-7) were performed to delineate and characterise language, cognitive and behavioural profiles in school-aged children with 22q11.2DS, 22q11.2Dup, 16p11.2DS and 16p11.2Dup.

8.1 Summary and discussion of main findings

8.1.1 Main findings in 22q11.2 CNVs

Study 1 - Which clinical, behavioural and neurodevelopmental features do patients with the 22q11.2Dup show? Which cross-sectional cognitive profiles and longitudinal cognitive trajectories do children with 22q11.2Dup demonstrate?

The first study (Chapter 2) was a retrospective chart review in 28 individuals with 22q11.2Dup. Frequent clinical symptoms encompassed nutritional issues (57%), failure to thrive (33%), transient hearing impairment (52%), and CHD (33%). While speech-language, developmental and motor delays were prevalent during infancy, attention (64%), learning (60%), motor (52%) and language (50%) difficulties mostly emerged during primary school years. ADHD was diagnosed in 44%. Median FSIQ fell within the borderline range (IQ 76), with 21% of individuals exhibiting mild ID. Longitudinal analysis of IQ scores revealed that nearly two-thirds (7/11) maintained a relatively stable

cognitive profile, while one-third (4/11) showed a growing into deficit trajectory, indicated by an IQ score at the second time point that was at least 10 points lower than the score at the first time point. Regarding language, three individuals displayed a relatively stable profile, one caught up with peers and two exhibited a growing into deficit trajectory. Speech-language therapy was received by nearly 86%, while half (52%) followed special education. Individuals with *de novo* duplications showed a trend toward experiencing more failure to thrive, whereas those with inherited duplications were more likely to attend special education.

Study 2 - Do parents report specific social-communicative challenges in children with 22q11.2Dup compared to their unaffected siblings, age-matched children with 22q11.2DS and typically developing peers?

The second study (Chapter 3) was a prospective study on social-communicative abilities in school-aged children with 22q11.2Dup (n = 19) compared to those of their unaffected siblings (n = 11) and age-matched children with 22q11.2DS (n = 19). Parents reported that both 22q11.2 CNV groups showed more social-communicative difficulties compared to the normative sample, whereas children with 22q11.2Dup seemed to occupy an intermediary position between their siblings and children with 22q11.2DS. Speech-language delays were observed in 79% (15/19) of 22q11.2Dup, while general communicative issues were reported in 47% (9/19). In comparison to 22q11.2DS, children with 22q11.2Dup were reported to demonstrate less frequent and less severe difficulties. In addition, parents reported more variable social-communicative outcomes, with reduced repetitive behaviours and restricted interests. Siblings of children with 22q11.2Dup showed milder social-communication challenges and similar variable profiles, suggesting that factors beyond the duplication itself, such as the broader genetic background, may contribute to social-communication profiles.

Study 3 - Which language profiles do children with 22q11.2Dup demonstrate compared to age-matched children with 22q11.2DS or typically developing peers?

The third study (Chapter 4) was a collaborative effort between two clinical genetics centres, CME-Leuven (Belgium) and CHOP-Philadelphia (USA) on language skills in school-aged children with 22q11.2Dup (n = 29), in relation

to age-matched children with 22q11.2DS (n = 29). Mean language skills were better in children with the 22q11.2Dup in comparison to those with 22q11.2DS, though the difference did not reach statistical significance. School-aged children with 22q11.2 CNVs experienced significantly more language problems in relation to the general population. Children with 22q11.2DS experienced language deficits starting at the word level, while the most encountered language difficulties of children with 22q11.2Dup started at the sentence level. Both receptive and expressive language in morphosyntactic and lexico-semantic areas were affected in 22q11.2 CNV populations.

8.1.2 Main findings in 16p11.2 CNVs

Study 4 - Do parents report specific social-communicative and behavioural challenges in children with 16p11.2 CNVs compared to children with the reciprocal CNV and the normative sample? Are communicative impairments associated with behavioural manifestations in the selected CNVs?

The fourth study (Chapter 5) was a collaborative multisite study between CME-Leuven (Belgium) and Geisinger ADMI (USA) on the prevalence, nature and severity of, and the association between behavioural and social-communicative features in 68 school-aged children with 16p11.2 CNVs. Compared to the general population, children with 16p11.2DS showed a high prevalence of social responsiveness and communication problems (69% for both), while approximately half (52%) displayed behavioural problems, including social, attention and thought problems, and being withdrawn/depressed. Children with 16p11.2Dup demonstrated even higher rates of social-communicative problems (73-90%) with statistically significantly more externalising and overall behavioural challenges (89%), including aggressive behaviours, and attention, social and thought problems. In both CNV groups, there was a strong positive association between overall behavioural and social-communicative skills.

Study 5 - Do children with 16p11.2DS show delayed developmental milestones, compared to typically developing peers? Which cross-sectional cognitive profiles and longitudinal cognitive trajectories do children with 16p11.2DS show?

The fifth study (Chapter 6) combined both prospective and retrospective data to investigate developmental milestones, cognitive profiles and longitudinal cognitive trajectories in 24 school-aged children with 16p11.2DS. Motor, language, and continence milestones were all delayed acquired. Average IQ was 71 with 46% (11/24) having FSIQ in the borderline range (IQ 70-84). Both intra- and interindividual variability were found across the five cognitive domains (verbal comprehension, visual spatial skills, fluid reasoning, working memory and processing speed) with significant discrepancies between verbal and nonverbal index scores in 55% (11/20). Longitudinal IQ-data indicated that school-aged children with 16p11.2DS performed statistically significantly poorer at the most recent time point ($p < 0.001$) with 58% demonstrating a growing into deficit profile.

Study 6 - Which language profiles do children with 16p11.2 CNVs demonstrate compared to the normative sample and unaffected siblings of children with 16p11.2DS?

The sixth study (Chapter 7) was a prospective study on language abilities of school-aged children with 16p11.2DS CNVs ($n = 31$) in comparison to the normative sample and unaffected siblings of children with 16p11.2DS ($n = 8$). The influence of non-verbal intelligence and autistic traits was investigated. Both 16p11.2 CNVs exhibited significantly poorer language skills compared to the normative sample and unaffected siblings of children with 16p11.2DS. No significant differences were found between children with 16p11.2DS and those with 16p11.2Dup. Severe language impairments were identified in 70% of individuals with 16p11.2 CNVs across all language subdomains (morpho-syntaxis and lexico-semantics), with both groups exhibiting significantly better receptive vocabulary skills than overall receptive language abilities. Expressive language deficits were more pronounced than receptive deficits only in children with 16p11.2DS. Non-verbal intelligence had an influence on language outcomes only in children with 16p11.2Dup.

8.1.3 Main findings across the four NDD-CNVs

The results of the six studies are summarised in Tables 8.2 and 8.3. An overview of the neurodevelopmental and behavioural phenotype associated with 22q11.2 and 16p11.2 CNVs is provided from both dimensional and categorical perspectives, based on average group scores and cut-off scores respectively. Unpublished data on IQ scores were added for the 22q11.2DS and 22q11.2Dup group, with a subgroup of 22q11.2DS previously published as part of the PhD of Dr. Ellen Van Den Heuvel (Van Den Heuvel et al., 2017; Van Den Heuvel, Jonkers, et al., 2018). Descriptive statistics for these unpublished data can be found in Table 8.1. Although all four NDD-CNV groups demonstrated heterogeneous profiles, this overview serves as a valuable tool for identifying neurodevelopmental and behavioural domains that need thorough assessment and intervention.

Table 8.2 indicates that school-aged children with 22q11.2Dup consistently exhibit less severe neurodevelopmental and behavioural outcomes compared to those with 22q11.2DS, whereas the opposite pattern is observed in children with 16p11.2 CNVs, with those with 16p11.2Dup showing more pronounced neurodevelopmental and behavioural challenges than those with 16p11.2DS. The percentages in Table 8.3 confirm these results on a categorical level. Significant cross-CNV differences were only found at the behavioural level for externalising and total behavioural problems in 16p11.2 CNVs (Chapter 5). Our findings further indicate that children with these four NDD-CNVs generally exhibit poorer neurodevelopmental and behavioural outcomes compared to the normative sample, apart from behavioural skills in children with 16p11.2DS.

Another consistent finding is that indirect language and communication measurements (standardised questionnaires) indicate better outcomes compared to direct measurements (standardised in-person assessments), implying that parents and caregivers may underestimate the extent of the difficulties their children experience, as was previously reported by Bennetts et al. (2016). This suggests that pragmatic language and speech impairments in these NDD-CNVs might be more severely impaired when assessed directly. It is important to note that direct measures are generally considered more objective than completing questionnaires, inherently involving a subjective interpretation (Bennetts et al., 2016). Bennets et al. (2016) revealed variable outcomes regarding the agreement between direct and indirect measurements in very young children, depending on the specific measurements used. Notably, they found the strongest agreement

between parent-reported and directly measured language skills for children with either poor or exceptional language skills. Bishop and Mcdonald (2009) emphasised that solely relying on language tests is not sufficient, advocating for the inclusion of parental reports to provide important complementary information during the diagnostic process. Overall, literature suggests a consensus that both direct and indirect assessment methods demonstrate strengths and limitations. Consequently, the integration of both approaches is widely recognised as a comprehensive and effective strategy for language assessment (Andrés-Roqueta et al., 2021; Bishop & McDonald, 2009; Ebert, 2017; Garibaldi et al., 2021; Torrens & Ruiz, 2021).

Table 8.1 – Descriptive data on IQ scores in 22q11.2DS and 22q11.2Dup from participants in Chapters 3 and 4.

	22q11.2DS FSIQ	**22q11.2DS NVI**	**22q11.2Dup FSIQ**	**22q11.2Dup NVI**
Chapter 3 - *N*	19	/	19	18
Mean (*SD*)	72.37 (14.76)		78.37 (12.95)	77.78 (16.60)
Median	74		82	75.5
Range	49 – 109		60 – 99	56 – 120
% with ID	47%		26%	42%
Chapter 4 - *N*	18	14	18	17
Mean (*SD*)	72.56 (18.07)	70.86 (13.29)	78.67 (13.26)	78.41 (16.89)
Median	77	68.5	82.5	76
Range	35 – 109	55 – 94	60 – 99	56 – 120
% with ID	39%	50%	26%	37%

Table 8.2 – Dimensional perspective on the neurodevelopmental and behavioural phenotype in 22q11.2 and 16p11.2 CNVs based on a clinical cohort.

	Subdomains	Based on these (sub)scores	22q11.2DS	22q11.2Dup	16p11.2DS	16p11.2Dup
Language and communicative skills	Language form	Syntax: CCC-2 Syntax	●	o	●	●●
		Syntax: CELF SST, RS, FS	● - ●●	●	●●●	● - ●●●
		Morphology: CELF WS	o	●	●●	●
	Language content	Semantics: CCC-2 Semantics	●	o	●	●
		Semantics: CELF CFD, SR	● - ●●	●	●●	●●●
		Vocabulary: CELF WC, EV, WD, PPVT	● - ●●●	o - ●	●●	●●●
	Language use	Pragmatic Language: CCC-2 PC	●	o	●	●●
	Comprehension	CELF RLI	●●	●	●●	●●●
	Production	CELF ELI	●●	●●	●●●	●●●
	Total Language	CCC-2 GCC	●●	o	●●	●●●
		CELF CLS	●●	●	●●●	●●●
	Speech	CCC-2 Speech	●●	●	●●	●●
(Social) behavioural skills	Social responsiveness	Social: SRS-2 SCI	●●	●●	●●	●●●
		Preoccupations: SRS-2 RIB	●●●	●●	●●	●●●
		Total: SRS-2 total	●●●	●●	●●	●●●
	Behaviour	Internalising: CBCL Int.	NA	NA	o	●●●
		Externalising: CBCL Ext.	NA	NA	o	●●●
		Total: CBCL total	NA	NA	o	●●●
Cognitive skills	Intelligence	Total: WISC-V FSIQ	●●	● - ●●	●●	●●
		Nonverbal: WISC-V NVI	●●	o - ●	●●	●●

o Normal, mean scores within the average range.

• Mild impairment: CELF – WISC-V – PPVT <85; CCC-2 GCC >104; CCC-2 PC >53; SRS-2 >60; CBCL >59; <-1 *SD* for subtests.

•• Moderate impairment: CELF – WISC-V – PPVT <78; CCC-2 GCC >110; CCC-2 PC >57; SRS-2 >65; CBCL >61; <-1.5 *SD* for subtests.

••• Severe impairment: CELF– WISC-V – PPVT <70; CCC-2 GCC >116; CCC-2 PC >59; SRS-2 >75; CBCL >63; <-2 *SD* for subtests.

NA, data not available.

Note. Blue refers to indirect assessments, while orange refers to direct assessments. Cut Abbreviations: CCC, Children's Communication Checklist; CELF, Clinical Evaluation of Language Fundamentals; SST, Sentence Structure (5.0–8.11 years); RS, Recalling Sentences; FS, Formulated Sentences; WS, Word Structure (5.0–8.11 years); CFD, Concepts and Following Directions (5.0–12.11 years); SR, Semantic Relations (≥9 years); WC, Word Categories-Receptive, -Expressive, and -Total, Expressive; EV, Expressive Vocabulary (5.0–8.11 years); WD, Word definitions (≥10 years); PPVT, Peabody Picture Vocabulary Test; PC, Pragmatic Composite; RLI, Receptive Language Index; ELI, Expressive Language Index; GCC, General Communication Composite; CLS, Core Language Score; SRS, Social Responsiveness Scale; SCI, Social Communication and Interaction; RIB, Restricted Interests and Repetitive Behaviours; CBCL, Child Behavior Checklist; Int., Internalising; Ext, Externalising; WISC, Wechsler Intelligence Scale for Children; FSIQ, Full-Scale Intelligence Quotient; NVI, Nonverbal Index.

Table 8.3 – Categorical perspective on neurodevelopmental and behavioural phenotype in 22q11.2 and 16p11.2 CNVs based on a clinical cohort.

	Subdomain deficits/delays: based on these (sub)tests		**22q11.2DS**	**22q11.2Dup**	**16p11.2DS**	**16p11.2Dup**
Language and communicative skills	Delayed language milestones*		95-96%	67-79%	91-92%	100%
	Language form	Syntax: CCC-2 Syntax	63%	42%	64%	73%
		Syntax: CELF SST, RS, FS**	55-76%	42-66%	80-91%	75-100%
		Morphology: CELF WS	50%	50%	75%	75%
	Language content	Semantics: CCC-2 Semantics	37%	42%	54%	40%
		Semantics: CELF CFD, SR	47-70%	57-63%	63-77%	50-100%
		Vocabulary: CELF WC, EV, WD, PPVT	57-90%	28-50%	62-92%	62-100%
	Language use	Pragmatic Language: CCC-2 PC	68%	37%	67%	80%
	Comprehension	CELF RLI	83%	70%	78%	90%
	Production	CELF ELI	92%	64%	91%	80%
	Total Language	CCC-2 GCC	79%	47%	69%	73%
		CELF CLS	83%	62%	96%	90%
	Speech	CCC-2 Speech	79%	58%	67%	80%
(Social) behavioural skills	Social responsiveness	Social: SRS-2 SCI	74%	47%	71%	90%
		Preoccupations: SRS-2 RIB	95%	47%	64%	90%
		Total: SRS-2 total	79%	47%	69%	90%
	Behaviour	Internalising: CBCL Int.	NA	NA	48%	78%
		Externalising: CBCL Ext.	NA	NA	29%	89%
		Total: CBCL total	NA	40%	52%	89%
Cognitive skills	Intelligence	ID (IQ <70):	39-47%	26-32%	38-43%	53-60%
Motor skills	Delayed motor milestones*		NA	58%	67%	NA

Motor skills	NA	52%	NA	NA

Note.

* Percentages for delays were calculated across most studies, resulting in a range of percentages. Information about developmental milestones, referring to the timely achievement of milestones across language and motor areas was gathered through clinical follow-ups or parental anamnestic reports.

** Percentages for certain language skills were based on several subtests of the CELF and PPVT, resulting in a range of percentages. For instance, the percentage on vocabulary is based on the subtests WC, EV and WD of the CELF and the PPVT total score.

Blue refers to indirect assessments, while orange refers to direct assessments. Cut-offs for deficits based on the manual cut-offs of the test instrument for summary scores: from mild problems on for CCC-2 GCC >104, CCC-2 PC > 53, CELF RLI – ELI – CLS <85, PPVT <85, SRS SCI – RIB – Total >60, CBCL Int. – Ext – Total > 59; from mild ID on for IQ <70; and <-1 *SD* for subtest scores: CCC-2 subtests Syntax – Semantics – Speech >13, CELF subtests SST – RS – FS – WS – CFD – SR – WC – EV – WD <7. Abbreviations: CCC, Children's Communication Checklist; CELF, Clinical Evaluation of Language Fundamentals; SST, Sentence Structure (5.0–8.11 years); RS, Recalling Sentences; FS, Formulated Sentences; WS, Word Structure (5.0–8.11 years); CFD, Concepts and Following Directions (5.0–12.11 years); SR, Semantic Relations (≥9 years); WC, Word Categories-Receptive, -Expressive, and -Total, Expressive; EV, Expressive Vocabulary (5.0–8.11 years); WD, Word definitions (≥10 years); PPVT, Peabody Picture Vocabulary Test; PC, Pragmatic Composite; RLI, Receptive Language Index; ELI, Expressive Language Index; GCC, General Communication Composite; CLS, Core Language Score; SRS, Social Responsiveness Scale; SCI, Social Communication and Interaction; RIB, Restricted Interests and Repetitive Behaviours; CBCL, Child Behavior Checklist; Int., Internalising; Ext, Externalising; ID, Intellectual Disability; NA, data not available.

8.1.4 Cross-domain comparison in the four NDD-CNVs

The main findings across the investigated neurodevelopmental and behavioural domains (see Table 8.2 and Table 8.3) will be described for each CNV in the following paragraphs.

Language, cognitive and social phenotype in 22q11.2DS

Overall, moderate language impairments were observed across both language comprehension and production, spanning various language domains, and manifesting even at the word level, consistent with previous research (Solot et al., 2019). Parents expressed particular concerns regarding their children's speech impairments, the inability to use contextual information and difficulties with coherence (Chapter 3), whereas direct assessment most commonly identified difficulties in defining words, formulating sentences and following oral directions (Chapter 4). These language skills were in line with their cognitive functioning, with 32-33% demonstrating borderline functioning and 39-47% showing mild-moderate ID, consistent with previous research (Campbell et al., 2022; De Smedt et al., 2007; Duijff et al., 2012; Fiksinski, Bearden, et al., 2022; Fiksinski, Heung, et al., 2022; Klaassen et al., 2016; Swillen et al., 2018). Consistent with other studies (Benedetti et al., 2021; S. J. R. A. Chawner et al., 2023; Clements et al., 2017; Jalal et al., 2021; Wenger et al., 2016), the majority of school-aged children demonstrated autistic traits, with restricted interests and repetitive behaviours reported in almost all cases. Therefore, the current clinical cohort appears to reflect the common neurodevelopmental and behavioural phenotype described in previous literature on 22q11.2DS.

Language, cognitive, social and motor phenotype in 22q11.2Dup

In Study 2 and 3 language and communicative skills were evaluated for the first time in a clinical cohort of school-aged children with 22q11.2Dup. Consistent with observations in 22q11.2DS, parents of children with 22q11.2Dup expressed concerns about their children's speech impairments, the inability to use contextual information and difficulties with coherence. At the qualitative level, the language and communicative skills of children with 22q11.2Dup are at an intermediary position between their siblings and children with 22q11.2DS (Chapter 3). Direct language assessments showed mild impairments across various language and communicative domains, alongside moderate expressive language impairments. Challenges in recalling sentences, following oral directions and understanding semantic relations or sentence

structures were the most commonly identified difficulties (Chapter 4). Their language skills were in line with their overall cognitive functioning (FSIQ) and non-verbal intelligence (Chapter 2). The average FSIQ fell within the borderline range (IQ 76-78), which is at the lower end of what has been reported previously (S. J. R. A. Chawner et al., 2021; Drmic et al., 2022; Jacquemont et al., 2022; Jalbrzikowski et al., 2022; Lin et al., 2020; Modasi et al., 2023; Modenato, Martin-Brevet, et al., 2021). Mild to moderate ID was present in 26-32%. Performance IQ exceeded verbal IQ in 35% (5/14), while another study reported a verbal-over-performance IQ discrepancy in 38% (3/8) (Drmic et al., 2022). Cognitive trajectories in 22q11.2Dup showed growing into deficit profiles in 36% (Chapter 2).

Autistic traits were prevalent in 29-47% with mean SRS scores in the clinical range, consistent with previous research (Lin et al., 2020; Wenger et al., 2016). A subset was diagnosed with a formal NDD diagnosis, such as ASD in 11-21% and ADHD in 21-44%. However, it is important to acknowledge that the true prevalence of neurodevelopmental and behavioural issues may be lower in less-biased cohorts, as suggested by results in carrier relatives (Drmic et al., 2022). Motor delays were common in infancy (58%), while gross and/or fine motor impairments were mainly reported at primary school age (52%) (Chapter 2). A subset was formally diagnosed with DCD, a feature not previously associated with 22q11.2Dup but commonly observed in individuals with 22q11.2DS (Cunningham et al., 2018; Óskarsdóttir et al., 2023).

Language, cognitive, social, behavioural and motor phenotype in 16p11.2DS

In early infancy, nearly all children experienced speech-language delays. By school age, severe core language impairments were ascertained in 70%, with significantly poorer expressive than receptive language skills. Children with 16p11.2DS showed significantly poorer language skills than their unaffected siblings, confirming previous findings (Hanson et al., 2015). Our study revealed generalised impairments across multiple language domains, consistent with previous research (Mei et al., 2018), rather than a syndrome-specific pattern affecting specific subdomains (Chapter 7). In line with results in 22q11.2DS (Chapter 3), parents were most concerned about the inability to use contextual information, difficulties with coherence and speech impairments (Chapter 5). Speech impairments might be partially explained by impairments in the basic mechanisms of speech motor control (Demopoulos et al., 2018). Direct assessments identified most encountered difficulties in defining words, recalling

sentences and explaining links between words. Language scores were lower than anticipated based on FSIQ and non-verbal cognitive functioning. Language impairments persisted even after controlling for autistic traits and non-verbal cognition (Chapter 7), mostly aligning with previous reports (Jiménez-Romero et al., 2022; S. H. Kim et al., 2020).

Average cognitive functioning fell within the borderline range, which is lower compared to previous reports (S. J. R. A. Chawner et al., 2021; Green Snyder et al., 2016; Hanson et al., 2015; Hippolyte et al., 2016; A. Moreno-De-Luca et al., 2015; Zufferey et al., 2012). Mild to moderate ID was present in 38-43% (Chapters 5, 6 and 7). In contrast with previous findings (Zufferey et al., 2012), a verbal over non-verbal discrepancy was noted in 40% of children, with both intra- and interindividual variability across cognitive indices. Longitudinal IQ-data revealed growing into deficit trajectories in 58% (Chapter 6), which is in line with results in 22q11.2 CNVs (Chapter 2). Overall, we found moderate deficits in social responsiveness, with internalising and externalising behavioural scores within the normal range, confirming previous studies and pointing to the high prevalence of autistic traits (Benedetti et al., 2021; Green Snyder et al., 2016; Hanson et al., 2015; A. Moreno-De-Luca et al., 2015; H. Smith et al., 2022). Consistent with findings in 22q11.2DS, behavioural skills were significantly associated with social-communicative skills, but mostly independent of level of cognitive functioning (Chapter 5).

Similar to 22q11.2Dup, achievement of motor milestones was delayed in 67% of children with 16p11.2DS (Chapter 6). This is consistent with previous research indicating persistent delays and deficits in motor coordination with high rates of DCD in 16p11.2DS (Bernier et al., 2017; Chung et al., 2021; Goldman et al., 2019; Hanson et al., 2015; A. Moreno-De-Luca et al., 2015; Qureshi et al., 2014; Taylor et al., 2021; Zufferey et al., 2012).

Language, cognitive and social, behavioural phenotype in 16p11.2Dup

While all children experienced some language delays in infancy, severe language impairments were observed in 70% of children in primary school, affecting both expressive and receptive language comprehension and production, and all language domains (Chapter 7). Consistent with observations in 22q11.2 CNVs and 16p11.2DS, parents were most concerned about the inability to use contextual information, difficulties with coherence and speech impairments (Chapter 5). Direct language assessments identified most frequently encountered

difficulties in defining words, recalling sentences and understanding semantic relations or sentence structures. Similar to 16p11.2DS, language skills were more impaired than their cognitive skills, although language outcomes were significantly influenced by cognitive outcomes (Chapter 7). Average cognitive functioning fell within the borderline range, which is lower compared to previous research (S. J. R. A. Chawner et al., 2021; D'Angelo et al., 2016; Green Snyder et al., 2016; Hippolyte et al., 2016; Taylor et al., 2023), with 53-60% showing mild-moderate ID. Overall, we found severe deficits in social responsiveness, with clinically elevated internalising and externalising behaviour, confirming previous research and pointing to the high prevalence of autistic traits (Green Snyder et al., 2016; S. H. Kim et al., 2020; H. Smith et al., 2022; Taylor et al., 2023). Parents predominantly reported aggressive behaviours, attention, social and thought problems (Chapter 5). Consistent with findings in 22q11.2DS and 16p11.2DS, behavioural and social-communicative skills were significantly associated, but independent of level of cognitive functioning.

8.2 Incomplete penetrance, variable expressivity and pleiotropy

A consistent finding across all six studies is the heterogeneous nature of the neurodevelopmental and behavioural phenotype in children with 22q11.2 and 16p11.2 CNVs. This heterogeneity is not unique but rather a common finding in the neurodevelopmental and behavioural phenotype of individuals with rare pathogenic variants, illustrating the concepts of reduced penetrance, variable expressivity and pleiotropy. These three phenomena present challenges in clinical practice and in research due to their complexity and the difficulty in elucidating their underlying mechanisms. The mechanisms by which they occur remain largely unexplored, though several theories have been proposed, such as the potential involvement of other uncommon genetic variations, polygenic risk factors, environmental influences, and the limitations of existing diagnostic criteria (Forrest & Penzes, 2023).

We hypothesise that neurodevelopmental and behavioural challenges stem from multiple contributing factors, which can vary significantly from one individual to another. In the introduction of this dissertation (Chapter 1), we provided an overview of potential contributing risk and protective factors and their accompanying impact on the neurodevelopmental outcome (see Figure 1.1). We will discuss these factors in the context of the current results of this dissertation and the existing literature: 1) individual-specific risk and protective

factors, 2) familial and environmental risk and protective influences, and 3) progression of time and development itself (de Voursney et al., 2008; Swillen et al., 2018). Apart from these factors, we acknowledge that other (genetic) factors, such as genetic modifiers, gene expression, and causal variants, might play a role. Since this was not the focus of this dissertation, more detailed discussion on the influence of genetic modifiers, gene expression and causal variants can be found in Kingdom and Wright (2022).

8.2.1 Individual-specific factors

Individual-specific factors may involve genetic, medical-biological and neurodevelopmental factors.

Genetic factors

A first genetic factor is the *presence of the specific CNV*. According to the current dissertation, 22q11.2Dup is associated with milder neurodevelopmental and behavioural phenotypes than 22q11.2DS, whereas 16p11.2Dup is associated more severe phenotypes than 16p11.2DS, which is in line with findings from two previous studies (S. J. R. A. Chawner et al., 2021; Gur et al., 2023). As both typical LCR22A-LCR22D and various nested and larger duplications were included (Chapters 2, 3 and 4), an additional factor contributing to variability may lie in *the size* and *positioning of the 22q11.2Dup*. However, earlier studies indicated that the size of the duplication may not reliably predict phenotypic expression (Dupont et al., 2015; Ensenauer et al., 2003; Portnoï, 2009).

Another genetic factor is the *mode of inheritance*: phenotypic outcomes of individuals with *de novo* and inherited CNVs were compared in all six studies, revealing no statistically significant differences. However, several qualitative differences were observed, including a higher prevalence of failure to thrive in *de novo* 22q11.2Dup, whereas more individuals with inherited 22q11.2Dup attended special education (Chapter 2). This discrepancy might have multifactorial causes in inherited CNVs, potentially stemming partially from assortative mating (see 8.2.2). Parents of children with 22q11.2Dup reported more diverse profiles on the social responsiveness composite scores in children with inherited duplications in contrast to those with *de novo* duplications (Chapter 3). On average, school-aged children with *de novo* 22q11.2Dup or 16p11.2DS had higher FSIQ scores than those with inherited duplications or deletions, respectively (Chapters 2, 6 and 7), which is consistent with studies in 22q11.2DS (De Smedt

et al., 2007; McGinn et al., 2022) and 16p11.2DS (D'Angelo et al., 2016; Pizzo et al., 2019).

Several studies have shown that *additional CNVs* are quite common in individuals with 22q11.2 and 16p11.2 CNVs (Girirajan & Eichler, 2010; D. Li et al., 2012; Newbury et al., 2013; Pizzo et al., 2019; Redaelli et al., 2019) and that these additional CNVs may contribute as genomic modifiers to phenotypic variations in 22q11.2DS/22q11.2Dup (D. Li et al., 2012) and lower cognitive skills in 16p11.2 CNVs (Hudac et al., 2020). Girirajan et al. (2012) observed significantly more pronounced phenotypes for affected individuals with CNVs or larger variants in addition to the 16p11.2DS. Finally, *the number of other hits*, defined as damaging variants in functionally intolerant genes, might modulate the severity of the phenotype, resulting in an additive or synergistic effect on neurodevelopmental pathways and disease outcomes, as it was previously associated with variable phenotypic traits in index patients with 16p12.1DS and 16p11.2DS (Girirajan et al., 2010; Oliva-Teles et al., 2020; Pizzo et al., 2019).

Medical-biological factors

The presence of certain medical comorbidities in relation to the phenotype was only considered in 22q11.2 CNVs, revealing no statistically significant effect of mild hearing loss, palatal defects or congenital heart disease on language outcomes in 22q11.2 CNVs (Chapter 4). Previous research showed that the severity of congenital heart disease or palatal defects/velopharyngeal dysfunction in children with 22q11.2DS is unrelated to variations in language or cognitive outcomes (De Smedt et al., 2007; Gerdes et al., 1999, 2001; Solot et al., 2001, 2019). Importantly, even mild hearing loss might influence language outcomes (Halliday et al., 2017; Lieu et al., 2020; Mei et al., 2018; Moore et al., 2020) and should be considered in CNV populations. This is particularly relevant given the high prevalence of transient or permanent hearing problems in 22q11.2Dup (Chapter 2), 22q11.2DS (Elden, 2022; Solot et al., 2019; Van Eynde et al., 2016; Verheij et al., 2017) and the lower prevalence observed in 16p11.2DS (Taylor et al., 2021). In 16p11.2 CNVs, the presence of clinical features such as epilepsy or overweight/obesity in relation to the neurodevelopmental and behavioural phenotype has not been investigated yet (Chung & Herrera, 2023; Moufawad El Achkar et al., 2022). In general, epilepsy can impact cognitive functioning, memory, language and frontal executive functioning skills (Hamed, 2009), whereas in individuals with ASD, epilepsy is associated with an elevated risk of language and cognitive impairments, although its influence on language

and cognition is still debated (Tuchman & Rapin, 2002). Overweight has been reported to be associated with lower cognitive and executive functioning skills in school-aged children and adolescents (Y. Li et al., 2008; E. Smith et al., 2011). Children with extreme prematurity (<32 weeks) were excluded in all studies of this dissertation to reduce the impact on neurodevelopmental and behavioural outcomes. Nevertheless, infants born moderate (32 - 34 weeks) to late (34 – 36 weeks) preterm could still experience poorer socio-emotional, cognitive and school outcomes and require greater special educational needs (Chyi et al., 2008; Talge et al., 2010). One study indicated that perinatal complications (e.g. low APGAR score, abnormal presentation, low birthweight, respiratory distress and/or preterm labour) contributed to more severe autistic traits in 16p11.2DS, but not in 16p11.2Dup. The lack of a perinatal influence in 16p11.2Dup might be associated with the reduced occurrence of perinatal events in 16p11.2Dup, the reduced severity of the phenotype observed in 16p11.2Dup and the limited sample size (Hudac et al., 2020). However, the current dissertation did not find evidence confirming a milder phenotype in 16p11.2Dup.

Sex could impact the expressivity of and penetrance of certain genetic variants (Kingdom & Wright, 2022). The influence of sex on the neurodevelopmental and behavioural phenotype was investigated across CNVs, revealing no statistically significant sex differences in 22q11.2 and 16p11.2 CNVs (Chapters 3, 4 and 6), in line with prior studies for 22q11.2DS and 16p11.2 CNVs (De Smedt et al., 2007; Green Snyder et al., 2016; Hanson et al., 2015; McGinn et al., 2022; A. Moreno-De-Luca et al., 2015). However, some studies on 22q11.2 DS indicated that males experienced more internalising and thought problems and had lower IQ scores compared to females (Niklasson et al., 2009; Sobin et al., 2009; J. A. S. Vorstman et al., 2006), while more neurocognitive impairments were observed in studies on 22q11.2DS with a higher proportion of male participants (Moberg et al., 2018). In one study, males with 16p11.2 or 22q11.2 CNVs had an elevated risk for ASD compared to females with these NDD-CNVs, although this difference is less pronounced than for idiopathic ASD (S. J. R. A. Chawner et al., 2021). Autism measures and clinical practices may be biased towards detecting autism in males, potentially contributing to these observed differences. Additionally, various NDDs exhibit a sex bias towards boys (Polyak et al., 2015; Werling & Geschwind, 2013). Consistent with these findings, the male-to-female ratio in both ASD and ID leans toward males in 16p11.2 CNVs (Polyak et al., 2015), indicating that being a girl might serve as a protective factor in predicting the severity of autistic traits, cognitive and adaptive functioning impairments in both 16p11.2 CNVs (Hudac et al., 2020). However, findings are

not consistent since one study found significantly higher non-verbal IQ in males than females for both 16p11.2 CNVs (D'Angelo et al., 2016), and sex did not significantly predict the risk/presence of psychosis in 16p11.2 CNVs (Jutla et al., 2020).

This dissertation did not investigate *cortical differences*, but they likely contribute to the variable phenotypic expressions observed. Previous neuroimaging studies have demonstrated substantial impact of 22q11.2 and 16p11.2 CNVs on global and regional brain volumes, cortical thickness and surface area with varying effect sizes (Ching et al., 2020; Dima et al., 2020; Gudbrandsen et al., 2020; K. Kumar et al., 2023; Maillard et al., 2015; Martin-Brevet et al., 2018; Modenato, Martin-Brevet, et al., 2021; Qureshi et al., 2014; Sun et al., 2020). Notably, gene dosage effects between duplications and deletions have been observed, resulting in opposing manifestations in brain volumes for both 22q11.2 (Lin et al., 2017; Seitz-Holland et al., 2021) and 16p11.2 CNVs (Y. S. Chang et al., 2016; Martin-Brevet et al., 2018; Owen et al., 2018). The cortical surface area was decreased in 22q11.2DS and increased in 22q11.2Dup compared to controls, with opposite patterns observed for cortical thickness (Lin et al., 2017; Seitz-Holland et al., 2021). Another study found opposing effects on white matter microstructure in 22q11.2 CNVs (Seitz-Holland et al., 2021). While 16p11.2DS correlated with increased brain volume, including macrocephaly, 16p11.2Dup was associated with deceased brain volume, potentially leading to microcephaly (Blackmon et al., 2018; Qureshi et al., 2014; Shinawi et al., 2010). These neuroanatomical changes linked with 22q11.2 and 16p11.2 share similarities with those seen in idiopathic ASD and Schizophrenia (Ching et al., 2020; Maillard et al., 2015; Martin-Brevet et al., 2018; Qureshi et al., 2014; Sun et al., 2020), which are highly prevalent in these CNVs. Until now, the associations between these neuroanatomical differences and language, cognitive or behavioural outcomes have not been studied widely. In 16p11.2DS, certain focal cortical abnormalities have been associated with lower expressive and total language outcomes in individuals between 5-21 years of age (Blackmon et al., 2018), whereas delayed magnetic mismatch fields were correlated with language and cognitive impairments in children aged 7-17 years with 16p11.2 CNVs (Matsuzaki et al., 2020). Structural-functional studies discovered changes in the structural connectivity of the brain, affecting both the ventral and dorsal language pathways in school-aged children (8-16 years) with 16p11.2DS, potentially explaining impaired overall, expressive and receptive language outcomes in this population (Ahtam et al., 2019; Berman et al., 2015).

Neurodevelopmental factors

The contribution of the level of cognitive functioning on other neurodevelopmental domains/outcomes was only explored in the studies with 16p11.2 CNVs. In 16p11.2DS, cognitive functioning was only significantly associated with the CCC-2 general communication outcomes. In both 16p11.2 CNVs, there was no statistically significant association between level of cognitive functioning and any other outcome. In addition, general linear models with FSIQ as covariate did not reach statistical significance in both 16p11.2 CNVs (Chapter 5). These findings are in line with previous studies in 22q11.2DS, where general cognitive skills demonstrated weaker correlations with language and behavioural outcomes (S. J. R. A. Chawner et al., 2023). These findings imply that intelligence may play a role in the presence of autistic preoccupations, communication, and behavioural difficulties in these CNVs, but does not fully explain their occurrence. This observation is also in agreement with previous (categorical) research, which suggested that ID and other NDDs represent pleiotropic manifestations of 22q11.2DS (Green et al., 2009; Niarchou et al., 2014). However, in 16p11.2Dup (Chapter 7) the opposite pattern was observed showing a significant influence of non-verbal cognition on language outcomes. Therefore, based on these findings cognitive skills seem to contribute significantly to the variability observed in language outcomes in school-aged children with 16p11.2Dup. Differences in sample sizes, used measurements and cognitive parameters (FSIQ versus NVI) could possible explain the differences in findings between chapter 5 and 7. Nevertheless, both 16p11.2 groups still showed lower language outcomes compared to the normative sample, after controlling for NVI (Chapter 7). These findings align with previous observations of more severe language impairments in 22q11.2DS in comparison to their non-verbal cognitive skills (Van Den Heuvel, Manders, et al., 2018).

The influence of the presence of comorbid NDDs such as ASD and ADHD on the neurodevelopmental and behavioural phenotype, was investigated for the four NDD-CNVs, revealing only a significant impact of ASD on language scores in 22q11.2DS (Chapter 4). This is in line with previous research indicating that lower language comprehension skills were associated with lower social responsiveness skills in children with 22q11.2DS (S. J. R. A. Chawner et al., 2023; Selten et al., 2023). However, social responsiveness skills were not predictive of language outcomes in 16p11.2 CNVs (Chapter 7). Similarly, comorbid diagnoses of ASD and ADHD did not result in different cognitive or language outcomes in 16p11.2DS and both 16p11.2 CNVs, respectively (Chapter 5 and 7), which

was only for 16p11.2Dup in agreement with previous findings (S. H. Kim et al., 2020). These results imply that although individuals with 16p11.2 CNVs commonly exhibit autistic traits, these traits might not be the main factors influencing their language skills.

8.2.2 Environmental factors

Environmental factors can influence the manifestation or severity of outcomes, either negatively or positively (Kingdom & Wright, 2022). As depicted in Figure 1.1, environmental risk factors may refer to various aspects such as low socioeconomic status (SES), low maternal education, low parental and sibling IQ, poor parenting style, parental stress, large household size, the absence of a social network and single-parent family structures (Chapter 1). While this dissertation mainly focused on individual-specific factors, we also investigated the influence of maternal education as a proxy for SES on neurodevelopmental and behavioural outcomes in 22q11.2 and 16p11.2 CNVs, revealing no significant differences after correction for multiple testing. Qualitatively, only children with 22q11.2DS from high SES backgrounds achieved higher language scores compared to children from middle SES backgrounds, consistent with previous findings (Allen et al., 2014; De Smedt et al., 2007; Fiksinski et al., 2018; Shashi et al., 2010). Furthermore, cohort-related differences across countries and native languages were explored in Chapters 4 and 5, revealing no statistically significant variations.

The familial context significantly shapes phenotypic manifestations in genetic variants and common diseases (Finucane et al., 2016). As discussed in Chapter 2, a positive family history for language, cognitive and behavioural problems could exacerbate neurodevelopmental outcomes in individuals with CNVs, adding to their genetic burden and potentially leading to more severe phenotypes. For instance, Pizzo et al. (2019) discovered that individuals with 16p12.1 CNVs and a strong family history exhibited more pronounced clinical presentations compared to those with a mild or no family history (Pizzo et al., 2019; Polyak et al., 2015). Moreover, the cognitive and social abilities of individuals with 16p11.2Dup, *de novo* 16p11.2DS, and 22q11.2DS are significantly associated with those of their parents (Fiksinski, Heung, et al., 2022; Klaassen et al., 2016; A. Moreno-De-Luca et al., 2015; Olszewski et al., 2014; Pizzo et al., 2019; Taylor et al., 2023). Neurodevelopmental abilities in individuals with these CNVs suggest an expected "shift" from anticipated outcomes, partially influenced by parental background across these neurodevelopmental domains. The degree to which an individual with a CNV reaches the threshold for a

diagnosable NDD can vary depending on the initial level of intelligence and social skills inherited from the parents (Finucane et al., 2016; D. Moreno-De-Luca & Martin, 2021).

Furthermore, assortative (non-random) mating may also contribute to differences in families with inherited CNVs compared to those with *de novo* occurrences. Assortative mating refers to the principle whereby individuals with overlapping phenotypic traits yet different underlying genotypes might choose to partner and produce offspring, resulting in the accumulation of genetic predispositions from both parents across successive generations (Smolen et al., 2023). For instance, individuals with inherited 22q11.2DS have lower FSIQ than individuals with *de novo* 22q11.2DS due to lower educational attainments of the parents of individuals with inherited 22q11.2DS. This may be attributed to the combination of the effect of the CNV (through the affected parent) and the principle of assortative mating (through the unaffected parent) (De Smedt et al., 2007). This phenomenon is not unique to 22q11.2 DS but also observed in several other CNVs and NDDs such as ASD, ADHD and schizophrenia. (Boomsma et al., 2010; Nordsletten et al., 2016; Plomin et al., 2016; Plomin & Deary, 2015).

A subset of individuals with 16p11.2 CNVs were adopted or stayed in foster care (Chapters 5, 6 and 7), indicating challenging circumstances that may pose a risk for neurodevelopmental outcomes (Berk, 2018). Although the literature lacks consistency, post-institutionalised international adoptees have been reported to exhibit more behavioural problems, particularly internalising, externalising and attention problems, if adopted after a stay of at 6 to 18 months in institutions such as hospitals, orphanages and baby homes (Hawk & McCall, 2010). This might be partially attributed to early deficient social-emotional caregiver-child interactions and fewer opportunities to form attachment relationships, consistent with the attachment theory (Hawk & McCall, 2010). In addition, children raised in families experiencing complex and multiple problems (FECMP) such as substance abuse, poverty, parental depression or parenting difficulties, are prone to developing difficulties across various life areas (van Assen et al., 2020; Visscher et al., 2020). Children leaving foster care often encounter challenges across multiple domains, including education, in comparison to their peers from the general population. However, stable foster care placements have been identified as relevant factors for improving outcomes (Gypen et al., 2017).

Other environmental and familial protective factors, such as therapy, effective schooling and an authoritative parenting style, may mitigate some of these challenges (Berk, 2018; Bush & Peterson, 2008; Singer et al., 2022). Many children with CNVs have attended special education programs and/or received speech-language therapy from early infancy on, which might have had a beneficial influence on their neurodevelopmental outcomes. Parental support networks have been identified as a protective factor (Butter et al., 2024; Fitzgerald et al., 2021; Gilmore, 2018). Nevertheless, positive and supporting environmental settings cannot completely compensate for genetic vulnerabilities (Karmiloff-Smith et al., 2012).

8.2.3 Time / development itself

The progression of time and development inherently plays an important role in shaping the evolving and variable neurodevelopmental outcomes observed in individuals with CNVs (Swillen et al., 2018). The results in Chapters 2 and 6 demonstrate similar findings across 22q11.2Dup and 16p11.2DS, indicating different cognitive trajectories across the lifespan with a subgroup displaying a growing into deficit trajectory with increasing age.

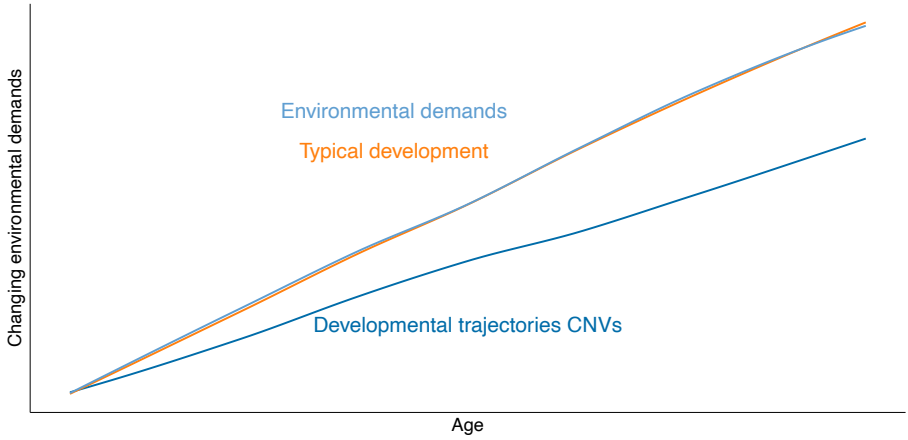

Figure 8.1 – Developmental trajectories in children with CNVs.
The orange line depicts an example of a typical developing trajectory, the light blue line refers to the altering environmental demands with age and the dark blue line represents an example of a typical CNV developmental trajectory. In the case of typical development, the lines of development and developmental demands nearly overlap. As children with CNVs grow older, the distance between their developmental level based on chronological age and the demands of their environment increases due to the neurodevelopmental and behavioural impairments associated with the genetic variant. Figure adapted from McDonald-McGinn et al., 2015 and Swillen, 2022.

This profile is often noted in children with CNVs (Figure 8.1), such as 22q11.2DS (McDonald-McGinn et al., 2015; Swillen, 2022; Swillen & McDonald-McGinn, 2015; Van Den Heuvel, Jonkers, et al., 2018) and might be in part due to the increased proportion of abstract reasoning abilities required in IQ tests with increasing age. Finally, the influence of age was investigated in 16p11.2 CNVs, revealing no significant impact on neurodevelopmental or behavioural outcomes (Chapter 5 and 6).

8.3 Methodological contributions and considerations

A key strength of the studies in this dissertation lies in the deep phenotyping approach (1.3) using valid instruments to comprehensively characterise the neurodevelopmental and behavioural features, across communicative, (social) behavioural, cognitive and motor domains, in school-aged children (5-17 years) with these specific NDD-CNVs. By integrating both a categorical and dimensional approach, we increased the understanding of the type, severity and impact of symptoms (1.3.1) during this age period. From a dimensional approach, these four CNVs act as potentially "shifting" neurodevelopmental and behavioural phenotypes on a spectrum from baseline values determined by a certain genotype. This challenges the conventional view that NDD-CNVs lead to specific categorical disorders in a specific proportion of carriers (Cable et al., 2021; Finucane et al., 2016). Using this combined approach, we observed in our studies (Chapters 5 and 7) that only a subset of school-aged children with 16p11.2 CNVs have a formal diagnosis of ASD and of language disorders (LD) while almost all present with autistic traits and language deficits, which has important clinical implications.

Incorporating both direct and indirect assessments (1.3.2) of language components expanded upon the rather limited information in the current literature, particularly in less-studied populations like 22q11.2Dup and 16p11.2Dup. While indirect methods, such as parental questionnaires, yielded valuable insights into the neurodevelopmental phenotype, they introduced potential biases due to varying interpretations and perceptions (Van Roy et al., 2010). Furthermore, our findings suggest that questionnaires often indicated milder language and communicative impairments compared to direct measurements, likely due to underestimation by parents and caregivers. Therefore, the combination of parental questionnaires with standardised in-person assessments is needed for a more comprehensive and objective evaluation of language abilities and impairments.

In addition, the use of standardised questionnaires and instruments enabled comparisons of results of individuals with NDD-CNVs with the normative sample. Using a between-group design (1.3.3), comparisons across the four NDD-CNVs allowed us to look for syndrome-specific features. The inclusion of unaffected siblings of children with NDD-CNVs (Chapters 2 and 7) provided insight into genetic and environmental background factors that may modulate neurodevelopmental outcomes. The three-tiered method (Chapters 3, 5 and 7) allowed us to analyse the outcomes from three different perspectives to better grasp the within-group heterogeneity (Olsson, 2005).

Cross-sectional research (1.3.4) is a good starting point for performing deep phenotyping in NDD-CNVs (Chapters 2-7). Nevertheless, the relationship between the genotype and phenotypic outcomes is not a straightforward one-to-one mapping, but rather a complex, time-dependent trajectory that requires longitudinal follow-up (Karmiloff-Smith et al., 2016). Therefore, we conducted a longitudinal study approach to characterise cognitive trajectories in 22q11.2Dup and 16p11.2DS (Chapters 2 and 6).

A methodological consideration is the absence of genetic testing in "unaffected" siblings, leaving open the possibility of undetected (subtle) genetic variants. Unfortunately, ethical and financial constraints prevented genetic screening for both small CNVs and larger genetic alterations in siblings. Another important consideration is that the sample size across all studies was still relatively small, because of strict inclusion and exclusion criteria and the rareness of these NDD-CNVs. Large intra-group variations resulted often in violation of assumptions and non-parametric testing, reducing the statistical power and calling for cautious interpretation of the results, especially considering the genetic-first approach.

Finally, it is important to acknowledge the presence of ascertainment bias inherent in clinical cohorts, which may skew findings towards more severe phenotypes and this should be considered when comparing results across different studies or regarding prevalence rates (Oliva-Teles et al., 2020). Therefore, the current findings of this school-aged NDD-CNV cohort cannot be generalised to the complete 22q11.2 or 16p11.2 CNV population. However, all research methodologies, including those labelled as "population-based", have inherent limitations and biases (Figure 8.2): each approach, whether clinical or population-based, offers unique insights and contributes to a shared goal of enhancing our understanding of diseases and mechanisms to ultimately improve

outcomes (Bassett et al., 2023). Therefore, clinical samples play a valuable role as an initial step in the process of identifying clinical syndromes or phenotypes (S. H. Kim et al., 2020).

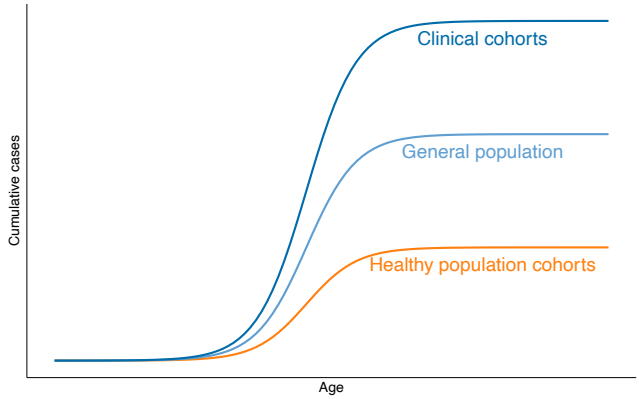

Figure 8.2 – Penetrance in clinical versus population cohorts.
The penetrance of genetic variants detected in clinical cohorts often appears higher compared to when those variants are ascertained in population cohorts. This difference may result in earlier disease onset, milder symptoms, or a greater number of affected individuals. Given the inherent ascertainment biases in both clinical and population cohorts, the true penetrance of variants in the overall unselected population is likely to fall somewhere between these two extremes. Figure adapted from Kingdom and Wright, 2022.

8.4 Clinical implications

Previously, genetic testing for most children was typically conducted after the emergence of behavioural and neurodevelopmental challenges, often resulting in the identification of an NDD-CNV. This contributed to a better understanding of the underlying phenotype (Figure 8.3). However, in recent years, there has been a shift towards earlier genetic testing, often identifying NDD-CNVs during infancy, before the onset of behavioural and neurodevelopmental difficulties. Early genetic testing can mitigate the uncertainty experienced throughout the diagnostic process and the accompanying relief felt upon diagnosis confirmation, often after years of anticipation (Kleinendorst et al., 2020). In addition, early detection presents an opportunity to explore potential prevention strategies (S. J. Chawner et al., 2021): for example, proactive screening for speech and language development could be initiated in children diagnosed with 16p11.2DS before speech and language challenges manifest. Bernier et al. (2017) reported that early motor milestones might predict ASD in 16p11.2DS, highlighting the important role of genetic

testing in customising assessment and intervention approaches for children facing language impairments and NDDs associated with specific genetic aetiologies (Selten, 2023). Moreover, there is increasing evidence suggesting that early interventions are beneficial for children with these conditions (Adams et al., 2013; Jiménez-Romero et al., 2022).

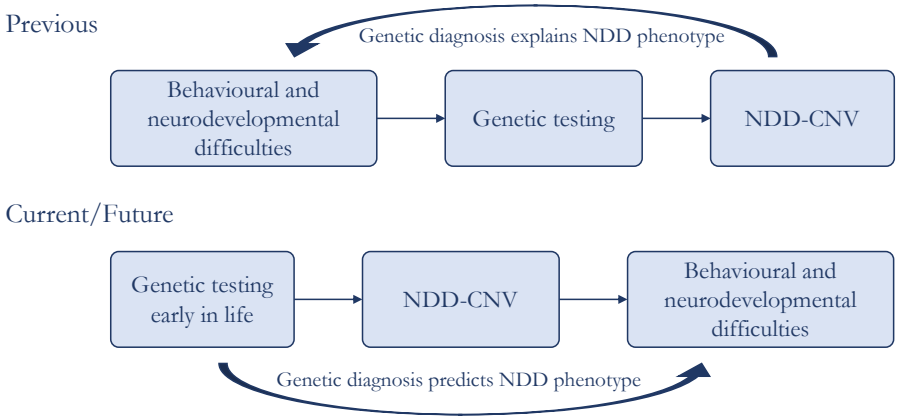

Figure 8.3 – Clinical genetics in neurodevelopmental and behavioural phenotypes.
Figure adapted from Perlman et al., 2023 and Vorstman et al., 2023.

8.4.1 Recommendations for assessment and follow-up

Given the increased risk for impaired outcomes across language, social, behavioural, cognitive and motor skills in children with 16p11.2 and 22q11.2 CNVs, combined with considerable variability, there is a need for targeted early neurodevelopmental monitoring and individualised multidisciplinary therapy. Achieving a balance between follow-up and support is crucial to adapt to the changing and increasing needs across the lifespan and to adjust environmental demands accordingly (Figure 8.1; Swillen, 2022; Swillen et al., 2018). Regular visits to the genetics clinic and/or the developmental paediatrician are valuable for addressing evolving questions and needs (Kleinendorst et al., 2020). In addition, it is recommended to visit specialised centres for clinical genetics and/or rehabilitation clinics since parents of children with NDD-CNVs feel that healthcare providers, teachers and educational professionals often lack knowledge and awareness of these conditions (Butter et al., 2024; Kleinendorst et al., 2020; Vo et al., 2018).

Due to the delays and difficulties present across multiple neurodevelopmental areas, a holistic and interdisciplinary approach is necessary

for both follow-up and intervention programs. This entails collaboration among various healthcare professionals (paediatrician, child psychiatrist, paediatric neurologist, clinical geneticists, genetic counsellors), therapists from different disciplines (speech-language pathologist, physio- and occupational therapist, and clinical educational psychologist) and caregivers (parents and teachers) to integrate assessments and interventions effectively (S. J. Chawner et al., 2021; Swillen, 2022; Swillen & McDonald-McGinn, 2015). During the evaluation and assessment, it is necessary to use norm-referenced, standardised tests due to their formal and decontextualised format, enabling comparisons to typically developing peers from the general population (Nicholls, 2018; Paul et al., 2018; Reed, 2018). Both indirect and direct assessments are recommended to combine different sources of information. Family members can give important insights into developmental milestones, communicative concerns and social, behavioural traits, providing a basis for the direction of evaluation (S. J. Chawner et al., 2021; S. H. Kim et al., 2020; Swillen, 2024; Tager-Flusberg et al., 2009).

As suggested in 16p11.2DS, it is recommended to conduct a comprehensive neurodevelopmental assessment at the time of NDD-CNV diagnosis, with subsequent re-evaluations at each health supervision appointment, preferably once per year (Chung & Herrera, 2023; S. Reilly et al., 2015) or at least during significant transition periods such as from preschool to primary and primary to secondary school. As language deficits are a core feature in 16p11.2 CNVs, language assessment is key (S. H. Kim et al., 2020), including both language comprehension and production of morpho-syntactic, lexico-semantic and pragmatic abilities, even in the absence of social or cognitive impairments (S. H. Kim et al., 2020; Solot et al., 2019). Supplementing standardised tests by analysing natural use of language in everyday situations, especially in children with ASD features, can provide valuable insights (Condouris et al., 2003; Jiménez-Romero et al., 2022; Reed, 2018; Tager-Flusberg et al., 2009). Moreover, language abilities should be interpreted in the context of cognitive abilities, which can be evaluated using standardised IQ tests such as the Wechsler scales (Swillen, 2024; Van Den Heuvel, Manders, et al., 2018). Nevertheless, the DSM-5 framework advises against solely relying on FSIQ scores (American Psychiatric Association, 2013). Instead, it underscores the importance of evaluating the broader cognitive profile, where a reliable and valid IQ serves as merely one component alongside adaptive functioning in diagnosing ID. When choosing the appropriate test, factors such as culture, language, communicative skills, environment, and chronological and developmental age should be considered (D. Moreno-De-Luca & Martin, 2021; Swillen, 2024). In

addition, healthcare professionals should interpret the cognitive skills of the child with an NDD-CNV in relation to those of first-degree relatives without the NDD-CNV (S. J. Chawner et al., 2021).

Being diagnosed with an NDD-CNV appears to hinder access to support, as it diminishes the willingness of clinicians to thoroughly investigate potential coexisting neurodevelopmental conditions (Butter et al., 2024). Neurodevelopmental conditions that occur simultaneously may remain undetected because symptoms are mistakenly attributed solely to a primary diagnosis. This phenomenon is often referred to as "diagnostic overshadowing" (Butter et al., 2024; Jones et al., 2008; S. Reiss et al., 1982). Since many children with 16p11.2 and 22q11.2 CNVs demonstrate certain neurodevelopmental features without meeting the criteria for a formal diagnosis, it is also important to look beyond DSM-5 diagnoses (S. J. R. A. Chawner et al., 2021). Beginning at age 5-6, it is recommended to initiate screening for attention problems, ADHD, autistic traits and ASD, with particular attention given to girls, who are frequently underdiagnosed (Hull et al., 2020; Niarchou et al., 2015, 2019). Delayed motor milestones, gross and fine motor problems, and coordination difficulties were prevalent in 22q11.2Dup and 16p11.2DS, with most individuals receiving physiotherapy (Chapters 2 and 6). These findings are in line with findings across 16p11.2Dup and 22q11.2DS (Bernier et al., 2017; Cunningham et al., 2018; Green Snyder et al., 2016), indicating the need for in-depth motor assessments and follow-up across these NDD-CNVs (Chung & Herrera, 2023; Cunningham et al., 2021).

Regular hearing screening is also advised due to the high frequency of recurrent ear infections and hearing loss (Chapters 2 and 4), which might adversely impact language development and academic performance (Daud et al., 2010; Halliday et al., 2017; Jiramongkolchai et al., 2016; Lieu et al., 2020). Given the increased risk of seizures/epilepsy in 16p11.2 CNVs, alertness for seizures and epilepsy is advised (Moufawad El Achkar et al., 2022). Potential signs of seizure activity should be discussed with the parents upon diagnosis of the CNV, whereas an electroencephalogram should be obtained if any signs of seizures are noticed (Chung & Herrera, 2023; Moufawad El Achkar et al., 2022; Steinman et al., 2016).

Environmental factors may impact the phenotype in children with NDD-CNVs, highlighting the importance of obtaining information on these during the assessment process (Swillen, 2024). Children of a parent who carries

an NDD-CNV might face an even greater risk of experiencing poorer outcomes, underscoring the need for intensified support and lifelong follow-up for these vulnerable families (Swillen, 2022; Swillen & McDonald-McGinn, 2015). Genetic counselling for such families should consider their available resources and may involve reaching out to other healthcare professionals, including social workers, to help identify community, social, financial and emotional support options (Chung & Herrera, 2023; Kleinendorst et al., 2020; Shashi et al., 2010).

8.4.2 Recommendations for intervention and therapy

The variable expressivity and reduced penetrance make it difficult to formulate general intervention strategies, applicable for the whole NDD-CNV population. In addition, limited evidence-based research exists on therapeutic interventions for children with NDD-CNVs and their effectiveness (S. J. Chawner et al., 2021; Solot et al., 2019). Many of the intervention strategies employed are derived from evidence-based methods used in children facing similar challenges without NDD-CNVs (Solot et al., 2019).

Language and communicative skills

Solot et al. (2019) propose effective intervention strategies for addressing speech and language impairments in individuals with 22q11.2DS, which may also apply to other NDD-CNVs. Due to the high incidence of speech-language delays in infancy (Chapters 2-7), interventions should start at the earliest opportunity to mitigate potential long-term effects on communication skills (Gladfelter et al., 2011; Solot et al., 2019). Speech-language therapy should be multifaceted and tailored to the individual's specific needs, strengths and weaknesses. It should equally address structural, semantic and pragmatic components of language. In addition, it should include strategies for enhancing communication and social interaction (Jiménez-Romero et al., 2022).

All school-aged children with the four NDD-CNVs (except for one with 16p11.2DS), were able to express themselves verbally. Nevertheless, persistent and significant language impairments present in these four NDD-CNVs warrant early referral for speech-language therapy upon diagnosis of the NDD-CNV, particularly in 22q11.2DS and 16p11.2 CNVs (Butter et al., 2024). During infancy and preschool, support should prioritise parental guidance, fostering communicative intentions, and improving social interactions, since communication problems often result in limited contact with peers (Kleinendorst et al., 2020; Solot, 2022; Solot et al., 2019). In cases of limited or absent verbal

output or severe speech sound disorders, a total communication approach is advised to support and facilitate the development of communicative skills. Certain children might benefit from the early introduction of visual alternative and augmentative communication (AAC) systems, either aided or nonaided (Jiménez-Romero et al., 2022; Solot et al., 2019). AAC systems enhance communication and language skills of individuals with neurodevelopmental difficulties (Jiménez-Romero et al., 2022; Millar et al., 2006; Reed, 2018). Nonaided AAC visual systems typically involve manual signs from sign languages, adapted for children with fine motor problems. Aided AAC visual systems encompass communication boards with pictures or symbols, such as the Picture Exchange Communication System, suitable for cases where significant language production impairment coexists with social communicative deficits (Jiménez-Romero et al., 2022; Reed, 2018; Solot, 2022; Solot et al., 2019). Bimodal communication, combining oral language with AAC, enhances communicative skills (Jiménez-Romero et al., 2022; Lesser & Ebert, 2020), by increasing verbal speech and social-communicative behaviours and reducing behavioural issues. In addition, it notably enhances comprehension of visual and oral instructions, along with contextual information in children with autistic traits or ASD diagnoses (Charlop-Christy et al., 2002; Ganz & Simpson, 2004; Jiménez-Romero et al., 2022; Santos et al., 2021).

During primary school age, language therapy should prioritise addressing impairments that significantly affect functional communication, academic achievement, and social interaction (Solot, 2022; Solot et al., 2019). As expressive language skills seem to be more impaired than receptive skills in children with 16p11.2DS, adopting case-based approaches with individualised, impairment-focused interventions might yield positive results (S. Reilly et al., 2015). Establishing the language domains for initial therapy objectives relies on identifying the individual needs of the child through the assessment process, guiding the initial focus on morphosyntactic, semantic and/or pragmatic abilities. For other children, immediate attention may be required for social interactive discourse. While intervention might initially emphasise one aspect of language over others, the focus and goals of intervention may evolve multiple times over a child's program, taking into account the other language aspects. For instance, practicing syntactic structures with unfamiliar words may not be very effective (Reed, 2018).

Given the important role parents play as active partners in intervention for young children, psychoeducation, recommendations and providing tools on

supporting effective communication in different situations is crucial (Reed, 2018; Van Den Heuvel et al., 2021). A meta-analysis (Donolato et al., 2023) revealed that oral interventions can improve language outcomes in children with NDDs. The study showed greater improvements in expressive language, particularly with longer sessions conducted over an extended period. This implies that condition-specific language interventions may not be necessary for NDD-CNVs. Instead, continuous and long-term support is generally required for most children with NDDs (Donolato et al., 2023). Table 8.4, adapted from Solot et al. (2019), outlines frequent communicative impairments along with suggested interventional approaches (Solot, 2022).

Table 8.4 – Frequent communication and language deficits in children with NDD-CNVs and interventional approaches

Communicative domain	**Frequent deficits in 22q11.2 and 16p11.2 CNVs and *suggested interventions***
Language content: - Lexicon - Semantics	Topic-based and/or abstract concepts or vocabulary - Lexicon: Challenges with terms with multiple meanings - Lexico-semantic: Superficial, concrete word/concept knowledge → *It is needed to teach vocabulary/concepts within all different contexts.*
Language form: - Morphology - Syntax	Development of syntax and sentence structures delayed - Morphology: Difficulties with irregular verbs/plural/exceptions in comparatives-superlatives - Syntax: Verbal output lacks complexity → *Syntax/Morphological exceptions might have to be taught directly in therapy (e.g. drive – drove – driven; foot – feet; good – better – best).*
Language use: - Use of context - Coherence	Difficulties with pragmatic language - Context: Difficulties with understanding humour, sarcasm and nonliteral use of language → *It is needed to explicitly explain and teach these.* - Coherence: Difficulties with getting the sequence of events when telling a story → *Assist with topic introduction, maintenance and shifting.*
Processing speed	The processing speed may be slower, leading to difficulties with comprehension of long, complex sentences → *Additional time and repetition might be necessary.*
Language in classroom - Understanding oral instructions - Short-term memory	Difficulties with following and remembering complex instructions → *Teachers may need to modify and simplify instructions, repeat instructions, and ensure instructions are understood.* → *Teachers may need to provide prompts to help the child transition from completing one task to starting another.* → *Teachers may need to provide scaffolding to assist the child with language production.*

Note. Table adapted from Solot et al., 2019.

Cognitive skills

The selection of the appropriate type of education for a school-aged child with an NDD-CNV should be based on the overall cognitive skills of the child with input from an educational psychologist. For some children, regular education with additional learning support and educational assistance may be suitable, while others might require more specialised educational trajectories along with individualised educational plans (IEP) tailored to their specific needs (Chung & Herrera, 2023; Kleinendorst et al., 2020; Óskarsdóttir et al., 2023; Swillen et al., 2018; Van Den Heuvel et al., 2021).

Based on clinical experiences in individuals with 22q11.2DS, Swillen et al. (2018) suggest strategies that could also benefit children with other NDD-CNVs. These strategies include structured learning environments, the use of concrete (visual) materials and hands-on experiences, an incremental approach involving repetition and practice, and a supportive learning atmosphere with explicit objectives and regular feedback. Guidance on effective learning strategies, including visualisation and mnemonic techniques, as well as preparatory instruction (pre-teaching) for new material, can also be beneficial (Swillen et al., 2018). In addition, environmental adaptations such as minimising auditory distractions, being seated near the teacher, providing structured desks, using visual or written timetables, and signalling activity changes through visual and verbal cues, providing a quiet area and allowing regular breaks can enhance learning (C. Reilly & Stedman, 2013; Solot et al., 2019).

When there is a change in the cognitive capacities of a child with an NDD-CNV, it is essential to establish realistic expectations and to tailor the learning environment accordingly. This ensures a harmonious balance between the individual's abilities and the environmental demands (home, school). By doing so, anticipatory guidance can be integrated both at home and in school, mitigating unnecessary stress (Swillen et al., 2018). Adjustments to therapy, such as shorter sessions, frequent breaks, and repeated content, should be considered (S. J. Chawner et al., 2021).

(Social) behavioural skills

The fact that many children with 16p11.2 and 22q11.2 CNVs demonstrate certain neurodevelopmental features of ASD without meeting the criteria for a formal diagnosis should be considered in intervention (S. J. R. A. Chawner et al., 2021). For instance, since autistic traits are prevalent (Chapters 3

and 5), children may be eligible for and find value in interventions commonly employed in the treatment of ASD, such as (individual) social skills courses or applied behaviour analysis (ABA). ABA therapy is personalised to address the specific behavioural, social, and adaptive strengths and challenges and is typically conducted in one-on-one sessions (Chung & Herrera, 2023; Eckes et al., 2023). However, research suggests that CNV carriers with ASD may derive less benefit from social skills training compared to individuals with ASD who do not have a CNV (S. J. Chawner et al., 2021; Tammimies et al., 2019).

The presence of social (interaction) difficulties among individuals with 16p11.2 and 22q11.2 CNVs (Chapters 2, 3, 5) from primary school years on can pose a significant challenge for these children, making them vulnerable to bullying (Mayo et al., 2019; Moss et al., 2022; Óskarsdóttir et al., 2023). Therefore, raising awareness and providing psychoeducation to parents and teachers on these vulnerabilities is needed. As part of proactive care, regular screening for social processing deficits should be conducted throughout the lifespan in children with NDD-CNVs (Swillen et al., 2018). Interventions should prioritise minimising social stress and improving social skills through socio-cognitive remediation programs (Glaser et al., 2012; Mariano et al., 2015) and/or cognitive-behavioural therapy (Mariano et al., 2015). Nevertheless, further research is needed to assess the long-term effectiveness of these strategies in children with NDD-CNVs.

As attention problems are prevalent (Chapters 2 and 5), children may benefit from structured learning environments without an overload of stimuli, using visual cues to improve sustained attention (Swillen et al., 2018). Consultation with a neuropaediatric specialist or child psychiatrist and/or behavioural specialist can assist parents in navigating suitable behavioural interventional approaches or obtaining prescription medications, including those for ADHD (Chung & Herrera, 2023; Taylor et al., 2021). Stimulants may alleviate attention problems, motor coordination, social interaction and pragmatic language outcomes (Rausch et al., 2017). For concerns related to severe aggressive or destructive behaviour in 16p11.2Dup (Chapter 5), consultation with a paediatric psychiatrist will be needed.

(Sensori)motor skills

As delayed motor milestones, gross and fine problems, and developmental coordination disorder (DCD) were prevalent in 22q11.2Dup and

16p11.2DS (Chapters 2 and 6), children may benefit from physiotherapy, occupational therapy, and sensory integration therapy from early age on (1-5 years) (Bernier et al., 2017; Chung & Herrera, 2023; Óskarsdóttir et al., 2023; Swillen et al., 2018).

8.5 Future perspectives

In section 8.1.3, we discussed the consistent finding that indirect language and communication measurements often indicate milder outcomes compared to direct measurements. This implied that parents and caregivers might underestimate their children's difficulties, and that pragmatic language and speech impairments should be evaluated by direct standardised assessments. Notably, research on pragmatic abilities in 22q11.2Dup and 16p11.2 CNVs is still limited, highlighting the need for further investigation in future studies (S. H. Kim et al., 2020). Despite the high prevalence of speech sound difficulties and disorders observed in 16p11.2DS and 22q11.2DS (Mei et al., 2018; Solot et al., 2019), these have not been thoroughly characterised through direct assessment in 16p11.2Dup and 22q11.2Dup and warrant further delineation.

In 16p11.2DS, Childhood Apraxia of Speech (CAS) is frequently diagnosed (Fedorenko et al., 2016; Mei et al., 2018; Raca et al., 2013). However, despite all children with CAS having previously seen a speech-language pathologist (SLP), only 29% had received a clinical diagnosis of CAS. This discrepancy suggests potential misdiagnosis within the clinical community and highlights a lack of targeted intervention for motor planning and programming deficits, crucial for improving outcomes in these children. The variability in diagnostic criteria for CAS among clinicians further complicates the issue (Mei et al., 2018). Therefore, it would be valuable to investigate if a similar prevalence of misdiagnosis exists in our Belgian cohort of 16p11.2DS and to explore any discrepancies between previous diagnoses and our findings. Additionally, it might be interesting to explore whether speech motor control difficulties and CAS (Demopoulos et al., 2018; Mei et al., 2018) are associated with fine and/or gross motor difficulties or diagnoses of DCD.

Because of the variable expressivity and reduced penetrance inherent in 22q11.2 and 16p11.2 CNVs, it is difficult to make long-term predictions regarding neurodevelopmental and behavioural phenotypes. Studies investigating early developmental milestones and factors predicting later outcomes are scarce. Additionally, published data on speech and language in adults with NDD-CNVs is still lacking, leading to limited knowledge and understanding of their

communication characteristics and needs. (Mei et al., 2018; Solot et al., 2019). Therefore, future studies on 22q11.2 and 16p11.2 CNVs should focus on phenotypic characterisation across the lifespan, with prospective and longitudinal data collection from early infancy through adulthood (Mei et al., 2018; Solot et al., 2019).

There is a need to further study the impact of individual-specific and environmental risk and protective factors (Butter et al., 2024; Swillen et al., 2018) on the outcome in individuals with NDD-CNVs. Genetic factors of interest include the size of the CNV, the inheritance pattern, parent-of-origin, the presence of additional CNVs or second hits (Girirajan & Eichler, 2010; McGinn et al., 2022). In addition, characterising medical comorbidities/burden in children with these NDD-CNVs, and their correlation with the neurodevelopmental outcome (motor, language cognition, behaviour) may inform approaches to school-related interventions and accommodations. Further investigation is warranted to determine whether environmental influences and parental outcomes affect the neurodevelopmental outcomes of individuals with 22q11.2Dup, similar to what has been observed in the three remaining NDD-CNVs (Fiksinski, Heung, et al., 2022; Klaassen et al., 2016; A. Moreno-De-Luca et al., 2015; Olszewski et al., 2014; Pizzo et al., 2019; Taylor et al., 2023). Intrafamilial phenotyping is important for accurately interpreting the impact of the NDD-CNV on neurodevelopmental outcomes (D. Moreno-De-Luca & Martin, 2021).

Future long-term outcome studies are necessary to investigate the efficacy and effectiveness of speech-language therapy and other interventions in NDD-CNVs (S. J. Chawner et al., 2021; Solot et al., 2019). Translational research is an important step toward enhancing our understanding of behavioural processes and advancing treatment to promote the overall well-being of children with NDD-CNVs (Karmiloff-Smith et al., 2016; Van Den Heuvel, 2016b).

Finally, in future research, it is crucial to explore the impact of dimensional traits in NDD-CNVs more deeply, as current literature still primarily focuses on categorical diagnoses. Utilising polygenic risk scores in individuals with NDD-CNVs shows promise for improving prediction accuracy and identifying those at highest risk of neurodevelopmental difficulties. However, translating these findings into clinical practice faces challenges such as limited genetic testing resources and a lack of standardised monitoring guidelines for individuals predisposed to NDD symptoms. The recently launched Genes to

Mental Health Network (G2MH) aims to address these challenges by collecting, sharing, and analysing comprehensive datasets that integrate genomics with dimensional measures of psychopathology across diverse populations and geographic regions (Jacquemont et al., 2022).

8.6 General conclusion

The current dissertation extended previous research on language, behavioural and cognitive profiles in school-aged children with 22q11.2DS, 22q11.2Dup, 16p11.2DS and 16p11.2Dup. We focused on cross-CNV comparisons within the same chromosomal region, intrafamilial comparisons with unaffected siblings and comparisons with the normative sample.

In the studies focusing on 22q11.2DS and 22q11.2Dup (chapters 2 to 4), a range of neurodevelopmental challenges were identified, including developmental delays, attention, learning, motor difficulties, and cognitive impairments. Notably, children with 22q11.2Dup showed intermediary social-communicative difficulties between their unaffected siblings and children with 22q11.2DS. While individuals with 22q11.2Dup generally demonstrated better language skills compared to those with 22q11.2DS, both groups experienced significant language deficits relative to the general population.

In the studies focusing on 16p11.2DS and 16p11.2Dup (chapters 5 to 7), high prevalence rates of social-communicative problems and behavioural challenges were observed in both CNV groups, with strong associations between these domains. Children with 16p11.2Dup exhibited more severe social-communicative difficulties and behavioural problems compared to those with 16p11.2DS. Additionally, delays in developmental milestones, cognitive impairments, and growing into deficit cognitive trajectories were noted in children with 16p11.2DS. Language impairments were prominent in both 16p11.2 CNV groups, with expressive language deficits being more pronounced in 16p11.2DS and non-verbal intelligence influencing language outcomes in 16p11.2Dup.

The six studies revealed heterogeneous profiles across all CNV groups, but with distinct patterns of neurodevelopmental and behavioural challenges. On average, children with 22q11.2Dup consistently exhibited milder outcomes compared to those with 22q11.2DS, whereas the opposite trend was observed in children with 16p11.2 CNVs, with more pronounced challenges in 16p11.2Dup. Additionally, the findings highlighted the need for direct assessments of language

and communication skills, as indirect measurements may underestimate the difficulties experienced by these children. Overall, these insights contribute to a deeper understanding of the phenotypic variability and clinical intervention considerations within these recurrent NDD-CNV populations.

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Personal Contributions

Jente Verbesselt was responsible for the design of the protocol of the six presented studies, for the recruitment of the participants, for the collection and analysis of the data, and for the writing of the manuscripts.

Conflict of Interest Statements

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List of publications

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Verbesselt, J., Van Den Heuvel, E., Breckpot, J., Zink, I., Swillen, A. (2023). Sociaal-communicatieve vaardigheden bij kinderen met 22q11.2 duplicatie, siblings en 22q11.2 deletie. Presented at the TOK-dag (Taalontwikkeling Kinderen in Nederland en Vlaanderen), Utrecht, 10 Nov 2023-10 Nov 2023. (professionally oriented)

Verbesselt, J., Andries, G., Herreman, I., Vanopstal, J. (2023). SpraakKlankOnderzoek (SKO) voor gevorderden. Presented at the Vorming voor Professionelen, Sig vzw. (professionally oriented)

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Scientific contributions

Jacquemont, S., Huguet, G., Klein, M., Chawner, S.J R A., Donald, K.A., van den Bree, M.B M., Sebat, J., Ledbetter, D.H., Constantino, J.N., Earl, R.K., McDonald-McGinn, D.M., van Amelsvoort, T., Swillen, A., O'Donnell-Luria, A.H., Glahn, D.C., Almasy, L., Eichler, E.E., Scherer, S.W., Robinson, E., Bassett, A.S., Martin, C.L., Finucane, B., Vorstman, J.A S., Bearden, C.E., Gur, R.E., Genes to Mental Health Network, , Geyskens, L. (contr.), Verbesselt, J. (contr.), Breckpot, J. (contr.) with Jacquemont, S. (corresp. author), Gur, R.E. (corresp. author) (2022). Genes To Mental Health (G2MH): A Framework to Map the Combined Effects of Rare and Common Variants on Dimensions of Cognition and Psychopathology. American Journal Of Psychiatry, 179 (3), 189-203. doi: 10.1176/appi.ajp.2021.21040432

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About the author

Jente Verbesselt was born in Bornem, Belgium, on the 2nd of May 1996. She pursued secondary education at Sint-Theresiacollege (STK) in Kapelle-op-den-Bos, where she studied Latin and Ancient Greek, earning an honourable mention at the 21st Flemish Olympiad Ancient Greek-Plato competition. Graduated from secondary education in 2014, she entered the Faculty of Medicine of KU Leuven, specialising in Speech-Language Pathology and Audiology Sciences. She earned her Bachelor's degree with great distinction (magna cum laude) in 2017, and her Master's degree with greatest distinction (summa cum laude) in speech-language pathology in 2018. Her Master's thesis, which introduced an innovative application for individual voice ergonomic risk assessment, received two awards for the overall process, scientific depth, and innovation. Continuing her academic journey, she went on a clinical internship to Audiological Centre Royal Kentalis in Amsterdam. She completed her Master of Science in Audiology with the greatest distinction (summa cum laude) in 2019. Her master's thesis, focusing on "Insight in variability for speech intelligibility in modulated noise with normal-hearing young adults", earned her the Outstanding Master Thesis award and the award for the student with the highest academic achievement.

In November 2019, she began her professional career as a speech-language pathologist at an outpatient rehabilitation centre CAR Brussel vzw. However, her passion for research in the domains of language, cognition, and behaviour in rare genetic disorders led her to pursue a doctoral journey. In January 2020, she commenced her doctoral training at the Laboratory for Behaviour and Neurodevelopment, affiliated with the Department for Human Genetics in cooperation with research group Experimental Oto-Rhino-Laryngology (ExpORL) within the Department of Neurosciences at KU Leuven. Under the supervision of Prof. Dr. Ann Swillen, Prof. Dr. Inge Zink and Prof. Dr. Jeroen Breckpot, she conducted doctoral research focusing on "Deep phenotyping of language, behaviour and cognition in school-aged children with recurrent copy number variants". She joined a professional working group and played an important role in the development of a new diagnostic tool for speech sound disorders, *SpraakKlankOnderzoek (SKO).* In February 2024, she was awarded a B.A.E.F. (Belgian American Educational Foundation) Fellowship, securing a post-doctoral position in the USA.

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